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Impacts of Zn-Spiked Sediments on Four Invertebrates: Implications for the Canadian Sediment Quality Guideline

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Canada

IMPACTS OF ZN-SPIKED SEDIMENTS ON FOUR BENTHIC INVERTEBRATES: IMPLICATIONS FOR THE CANADIAN SEDIMENT QUALITY GUIDELINE

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SUMMARY

To assist in the development of the Canadian Sediment Quality Guideline (SQG) for Zinc (Zn) from its interim status to full status, spiked sediment toxicity tests were conducted with various sediment types with four freshwater aquatic invertebrate species, *Hyalella azteca* (amphipod), *Chironomus riparius* (midge), *Hexagenia* spp. (mayfly) and *Tubifex tubifex* (Oligochaete worm). The changes in Zn bioavailability in relation to different sediment types as well as different overlay water types could have implications on the SQG. This work was undertaken to produce spiked sediment toxicity test (SSTT) results with a wider range of sediment types. In the past, published SSTT results for Zn reported the onset of toxicity at concentrations higher than the current Interim SQG (ISQG), which was derived based on the co-occurrence approach.

The objectives of the study were to: 1) fill data gaps for the development of the Zn SQG from interim to full status, 2) assess Zn toxicity as a function of sediment characteristics, 3) compare species sensitivities to Zn, 4) assess overlying water hardness and volume on sediment toxicity, and 5) examine Zn bioaccumulation patterns. Test species were exposed to sediments from Lake Erie, representing a pelagic sediment (LE303), and a marsh sediment (Long Point) from a hard water lake, and Lake Restoule (Restoule), representing a sediment from a soft water lake on the Canadian Shield. Toxicity tests were performed with a hard overlay water and the *H. azteca* tests were also performed with a soft overlay water.

Organic carbon in sediments ranged from 5 to 110 mg•g^{-1} dry wt., the clay content ranged from 6 to 52 %, silt ranged from 48 to 75 % and sand ranged from 0.2 to 24 %. The LE303 Zn-spiked sediment, which had the lowest organic carbon content and a intermediate clay content, was up to four times more most toxic than Long Point and three times more toxic than Restoule sediments when the test were done in hard water. The least toxic was the Zn-spiked sediment from Long Point, which had the highest organic and sand content and the lowest clay content. Restoule sediment, which had a high organic content, the highest clay content and the highest load of metals, of which 8 exceeded federal or provincial SQGs, was more toxic with hard overlay water than Long Point sediment with hard overlay water but was less toxic than LE303 sediment with hard overlay water. The Zn-spiked Lake Restoule sediments were the most toxic when tested with soft overlay water such that there was no survival of *H. azteca* at any Zn concentrations including the controls. However, increased water to sediment ratio improved *H. azteca* survival; the LC50 at a 500:1 ratio of soft water to sediment was four times lower than that with hard water. Therefore, soft water shield lakes of Canada are likely much more sensitive to Zn contamination.

The relationship between biological effects (survival, growth and reproduction) and sediment, water and tissue residue concentrations were compared for each organism. In general, growth was the

most sensitive endpoint for *C. riparius* and *H. azteca*, but it was variable. Survival between these two species was similarly sensitive and this endpoint was generally less variable. Overall, *C. riparius* and *H. azteca* demonstrated similar sensitivities. Both *Hexagenia* spp. and *T. Tubifex* were less sensitive than the other two species and their most sensitive endpoints were growth and reproduction, respectively.

Increased water hardness was protective to *H. azteca* such that the critical concentrations were higher. There was an increased release of Zn from the sediment into the soft overlay water such that Zn water concentrations were as much as 10 times higher than in the hard water. There was very little difference in the release of Zn to the overlay water across a range of water:sediment ratios except in soft water experiments with Lake Restoule. However with increasing water:sediment ratio there was improved water quality (i.e. stable neutral pH, lower ammonia levels and stable saturated oxygen levels).

The current ISQG and probable effect level (PEL) for Zn, are 123 and 315 ug•g^{-1} dry wt., respectively (CCME, 1999). The lethal concentration LC10 or the effect concentration EC10, are proposed for use in the calculation of the SQG, in replacement of the lowest observed effect concentration (LOEC) or the effects range low (ERL), which were used in the determination of the ISQG and the PEL. The LC10 and EC10 were calculated from the single species toxicity tests and represent the concentration at which there was 10% mortality and a 10% decrease in sub-lethal endpoints (e.g., growth), respectively. From the current work, the LE303 Zn-spiked sediment was the most toxic and *C. riparius* was the most sensitive organism. The EC10 and EC20, determined for *C. riparius* in LE303 sediment, was 80.0 and 110 ug Zn•g-1 dry wt sediment, respectively; both values were lower than the current ISQG. Additionally, the EC50 of 192 ug Zn•g-1 dry wt sediment as well as the LC10 and LC20 of 269 and 304 ug Zn•g-1 dry wt sediment, respectively, were lower than the PEL, while the LC50 of 380 ug Zn•g-1 dry wt sediment was higher than the PEL. The LC10, LC20, EC10 and EC20 for *H. azteca* with LE303 sediments were less than two times the PEL and the EC10 for *Hexagenia* spp. growth was also less than two times the PEL. This research has provided SSTT data on four benthic invertebrates that can be used in species sensitivity distributions as well as in the calculation of a full SQG and has also provided some insight into factors that can affect Zn bioavailability and toxicity.

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1 INTRODUCTION

The majority of Canadian Sediment Quality Guidelines (SQGs) were approved in 1995 and published as the Canadian Environmental Quality Guidelines by the Canadian Council of Ministers of the Environment (CCME, 1999). Development of the SQGs was based on two approaches: co-occurrence field data and spiked sediment toxicity tests (SSTT). A SQG was considered “interim” (ISQG) if only one of the two approaches was used to calculate the value. At that time there was insufficient published SSTT data for all metals including zinc. Therefore, an ISQG for zinc was developed based on the co-occurrence approach using a modification of the National Status and Trends Program (CCME 1999). The current ISQG for Zn is 123 $ug \bullet g^{-1}$ dry wt. and the probable effects level (PEL) is 315 $ug \bullet g^{-1}$ dry wt. (CCME, 1999). The Water Quality Task Group has identified that the lack of SSTT data for zinc prevented the development of a full SQG since it could cause problems in the interpretation and implementation of the guideline. At the time of the development of the Zn ISQG, there were very few published SSTT results for Zn. Borgmann and Norwood (1997) in their spiked sediment test reported the onset of toxicity to *Hyalella azteca* at concentrations higher than the ISQG with a Lowest Observed Effect Concentration (LOEC) of 4,576 $ug \bullet g^{-1}$ dry wt.. This LOEC was 15 and 37 times higher than the current PEL and ISQG respectively. The spiked sediment used in the Borgmann and Norwood (1997) study (collected from Hamilton Harbour, Ontario), consistently supported high survival (*H. azteca*) in 4-week chronic toxicity tests and was “rich” with fairly high organic content as indicated by a Loss on Ignition (LOI) of 9.1% of dry mass. This example therefore was only relevant for *H. azteca* and was complicated by high organic carbon levels in sediment whereas the ISQG was developed to protect all benthic organisms across all ecosystems.

Therefore, the main goals of this study were to: 1) fill data gaps for the development of a SQG; 2) assess modifying factors in the sediment that affect toxicity; 3) assess overlying water hardness and volume on sediment toxicity; 4) determine the sensitivity of different species to Zn, and: 5) examine Zn bioaccumulation patterns. A variety of sediments with a range of organic content, particle size and from different ecosystems were selected for spiking. As well, different water to sediment ratios in the tests were compared, different overlay water hardness was investigated. Four test species representing different taxonomic groups, different life styles and different feeding strategies were selected for testing. Overall, these results should help determine the influence of organic carbon, sediment grain size, overlaying water hardness and biological factors (different organisms) to zinc bioavailability and toxicity and thus be useful in the further development of a sediment quality guideline for Zn.

2 MATERIALS AND METHODS

2.1 Sediment Collection and Preparation

Three different sediments were collected to provide a range in total organic carbon (TOC) concentration. Two sediments were collected from Lake Erie: 303 (Lat. 42.5641, Long. -80.0411) and Long Point (Lat. 42.5869, Long. -80.4522). One sediment was collected from Lake Restoule (Lat. 46.0569, Long. -79.8061), located on the Canadian shield near North Bay, Ontario. From this point on the Lake Erie site 303 will be referred to as LE303, the Lake Erie Long Point as Long Point and the Lake Restoule as Restoule. Sediments were collected with a mini box corer. Approximately 7 L of the top 10 cm was collected and placed in white polyethylene buckets with snap down lids. Excess water was decanted off of the sediment before storing at $4^{o}C$.

2.2 Sediment Spiking

The spiking procedures were a modification of Milani *et al.* (1996). The amount of metal stock spiked into the wet sediment was based on the dry weight fraction of the sediment in order to achieve the final concentrations series. The volume of sediment required at each zinc concentration for all test organisms, including replicates was determined. A maximum spike concentration, based on best available information on the toxicity of the metal to the same or similar species (usually at least two times the LC50) was determined. Initial estimates of Zn concentrations needed to cover the toxic range were 0, 180, 320, 560, 1000, 1800, 3200, 5600 and 10000 ug/g for the fine grained, silty and low TOC LE303 sediment and the coarser grained with high TOC Long Point sediment (Table 1). Restoule sediments had a high TOC concentration as well as a high percentage of the fine grained clay fractions (Table 1) and therefore the following higher Zn concentrations were used: 0, 320, 560, 1000, 1800, 3200, 5600, 10000 and 18000 ug/g.

The spiking solution was made in de-ionized water (Nano-pure) to a volume equivalent to the amount of sediment to be spiked (e.g. 1L of Zn solution for every 1L of sediment). A relatively large volume of spiking solution reduces the Zn concentration required in the solution and therefore reduces the likelihood of rapid precipitation of Zn when mixed with sediment, thus ensuring a more even distribution of Zn throughout the sediment. The mixing container was marked with the volume of sediment required and the total volume of sediment plus spiking solution. The appropriate spiking solution was added to the container and then sediment was added until the volume was displaced to the total volume top mark, thus a 50:50 mix of sediment and spike solution. The slurry was then mixed with a 16 inch double shaft, Double Helix mechanical mixer (Model DH-050-5N) powered by a 1000 watt variable speed electric drill. The spiked sediment sample was allowed to settle until the sediment volume was equal to the original volume of sediment spiked (approximated 2-3 weeks) then the overlying water layer was

decanted. If a flocculent or poorly differentiated layer formed between the solid sediment and overlying water, it was suctioned off into smaller containers and centrifuged at 3,200 R.P.M for 30 minutes. The supernatant was decanted and the remaining sediment was added back to the main sediment sample and mixed thoroughly. The process repeated as necessary until the final sediment volume was equal to the original sediment volume. All spiked sub-samples were combined and mixed, placed into high density polyethylene (HDPE) containers with no headspace and aged for approximately 6 months in the dark at 4±2°C to allow equilibration of the Zn and other chemicals in the sediment mixture (Environment Canada, 1997). The 6 month aging was longer than the recommended 4 weeks (Environment Canada, 1995), but occurred due to test scheduling restrictions.

Prior to sediment spiking, any visible indigenous organisms and large debris were removed using forceps, and any water sitting on the sediment surface due to settling of the sediment during storage, was mixed back into the sediment. After the LE303 sediment had been spiked with Zn and the toxicity tests initiated, it was discovered that this sediment contained a large population of oligochaete worms. Consequently, the Long Point sediment (also from Lake Erie), was sieved by rinsing the wet sediment through a clean stainless steel sieve (250 µm - #60) with Burlington municipal tap water originating from Lake Ontario, to remove the small indigenous worms and other biota before spiking. This was repeated for a number of batches until enough sediment was sieved. The sieved sediment and rinse water were allowed to settle for a minimum of 24 hours and then the excess rinse water decanted. All sediment batches were recombined and mixed thoroughly then stored at 4°C in the dark until use. The Restoule sediment did not contain any significant numbers of benthic invertebrates and therefore was not sieved. Normally, field sediments are not sieved prior to testing since the sieving process could change the natural state of the sediment by diluting out ions and other dissolved material. However when significant indigenous populations of organisms that are indistinguishable from the test organism are present, sieving is recommended.

2.3 Tissue, Sediment and Water Analyses: Physical and Chemical Analysis

Prior to spiking, a sub-sample of each sediment was collected for physical and chemical analysis. Approximately 50 ml of sediment was placed in HDPE containers for freeze drying, grain size measurement (sedigraph and sieve methodologies), organic and inorganic carbon analyses and loss on ignition (LOI) analysis by Environment Canada's Sedimentology Laboratory (Burlington, ON), or by the Ministry of the Environment (MOE) analytical laboratory (Etobicoke, ON). As well, a sub-sample of dried sediment were digested with nitric acid, hydrochloric acid and hydrogen peroxide (9:2:1 ml) at 200°C in a high pressure microwave oven for 15 minutes, cooled and brought to 100 ml volume with deionised water. The resultant solution was analyzed for Hg by Cold Vapour Atomic Absorption

Spectrometry (CVAAS) for 18 other elements (Al, As, Ba, Be, Cd, Co, Cr, Cu, Fe, Mn, Mo, Mg, Ni, Pb, Sr, Ti, V, and Zn) by Inductively Coupled Plasma - Mass Spectrometry (ICP-MS). and Total-P by the acidic persulfate digestion, automated continuous segmented flow analyzer, stannous chloride-molybdate complex, photometric method. Analyses were performed at Environment Canada's National Laboratory for Environmental Testing (NLET) (Burlington, ON) or by the MOE's analytical laboratory.

Samples of overlay water were collected at the beginning and end of every test and analyzed for Zn, pH, total ammonia, oxygen and conductivity. The samples for Zn analyses were preserved with nitric acid and analyzed by graphite furnace atomic absorption spectrophotometry (GFAAS). Samples of the DeChlor culture and bioassay water were collected from December 2005 through to April 2007 for analyses of dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), chloride (Cl) and sulphate (SO_4). Also, overlay water samples were collected from experiments with Restoule and analyzed for 35 elements (Ag, Al, As, B, Ba, Be, Bi, Cd, Ce, Co, Cr, Cs, Cu, Fe, Ga, La, Li, Mn, Mo, Nb, Ni, Pb, Pt, Rb, Sb, Se, Sn, Sr, Ti, Tl, U, V, W, Y and Zn) by Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) to determine the effect of the Zn spike and hardness on the release of these elements from the sediment.

Dry invertebrate tissues were cold digested with nitric acid and hydrogen peroxide and analyzed for Zn by GFAAS. Also, dried sub-samples of sediment, collected at test set-up, were also analyzed in the same manor.

2.4 Toxicity Tests

Four chronic toxicity tests were performed to determine the toxicity of the Zn-spiked sediments: a 10-day survival and growth test with *Chironomus riparius*, a 28-day survival and growth test with *Hyalella azteca*, a 21-day survival and growth test with *Hexagenia* spp. and a 28-day survival and reproduction test with *Tubifex tubifex*. *H. azteca* was tested with all three spiked sediments, *C. riparius* and *Hexagenia* spp. were tested on LE303 and Long Point spiked sediments and *T. tubifex* was tested on Long Point sediment. Details of toxicity test methods are described in Borgmann *et al.* (2005), Reynoldson *et al.* (1991, 1998) and Bedard *et al.* (1992). Static toxicity tests were conducted in aerated 250 mL glass beakers (*C. riparius*, *T. tubifex*), 1 L glass jars (*Hexagenia* spp.), and 1 L polycarbonate Imhoff settling cones (*H. azteca*). All tests were conducted with de-chlorinated (DeChlor) tap water from Lake Ontario (hard water). A second sets of tests with *H. azteca* were conducted with the Restoule sediments with 10% DeChlor (90% de-ionized water). All the test organisms were cultured in the 100% DeChlor water. Ratios of water to sediment were 10:1 by volume for all tests except *H. azteca*, which used a 67:1 ratio for all sediments and the additional ratios of 200:1, 500:1 and 1000:1 with the Restoule sediments, and one *Hexagenia* spp. test with LE303 sediments which was run at a 4:1 ratio. The aerated

water and sediment were allowed to equilibrate (in the environmental chambers in the dark) for 2 weeks prior to initiation of toxicity tests since it has been reported that a "quasi-equilibrium" is established after an initial 1-2 week period (Borgmann and Norwood, 1999).

Four replicates for each treatment and 6 replicates for the control were set up for all organisms except *Hexagenia* spp., which had 3 replicates for all treatments. Dissolved oxygen, pH, conductivity, temperature, and total ammonia were measured at the start (day 0 – prior to the introduction of organisms), and the completion of the test in every treatment. Evaporated water (approximately 1%) was replaced with de-ionized water at each feeding. Tests were run in an environmental chambers kept at 25 ±1°C (*H. azteca*) or 23 ±1°C (*C. riparius, Tubifex* & *Hexagenia* spp.), under a photoperiod of 16L:8D and an illumination of 500–1000 lux, with the exception of *Tubifex* test, which was run in the dark.

For a test to be acceptable, survival in a reference or laboratory control sediment had to exceed specific minimum levels: 80% for *H. azteca* and 70% for *C. riparius* (USEPA 1994; ASTM 1995); 80% for *Hexagenia* spp., and 75% for *T. tubifex* (Reynoldson *et al.*, 1998). Brief descriptions of each test are provided below.

2.4.1 Hyalella azteca chronic (28-day) survival and growth test

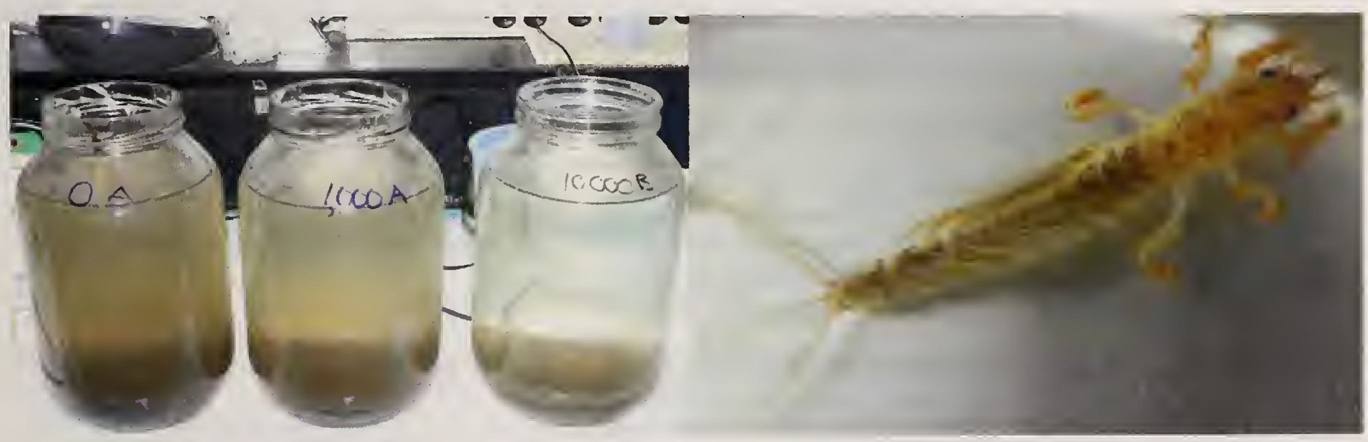
The *H. azteca* test was conducted for 28 days using 0-7 day old organisms (15 organisms per replicate cone). Each cone was Tetra-Min® fish flakes at a rate of two, 2.5 mg in weeks 1 and 2, three 2.5 mg in week 3, and one 5 mg in week 4. On day 28, the contents of each cone were wet sieved through a 250-µm screen and the surviving *H. azteca* counted. The animals were then gut cleared in a 50 umol EDTA solution for 24 hours. The gut clearing beakers contained a 5 by 5 cm piece of 100% cotton gauze and the organisms were fed 2.5 mg. After gut-clearing, the organisms were weighed, dried at 60°C for 72

hours, reweighed and placed in cryovials. Endpoints included percent survival and growth (initial weights of organisms were considered negligible).

2.4.2 Chironomus riparius chronic (10-day) survival and growth test

The *C. riparius test* was conducted for 10 days using first instar organisms (15 organisms per replicate beaker). Each beaker was fed 8 mg Nutrafin® fish flakes three times throughout the test. On day 10, the contents of each beaker were wet sieved through a 250-µm screen and the surviving *C. riparius* counted. *C. riparius* were then sediment gut cleared in a 50 umol EDTA solution for 24 hours. The gut clearing beakers contained a monolayer of silica sand and the organisms were fed 8 mg test food. After gut-clearing, the organisms were weighed, dried at 60°C for 72 hours, reweighed and placed in cryovials. Endpoints included percent survival and growth (initial weights of organisms were considered negligible)

2.4.3 Hexagenia spp. *chronic (21-day) survival and growth test*



The *Hexagenia* spp. test was conducted for 21 days using 10 pre-weighed mayfly nymphs (5 - 8 mg wet weight/nymph) per jar. Each jar was fed 40 mg Nutrafin fish flakes twice per week. On day 21,

the contents of each jar were wet sieved through a 500-µm screen and surviving mayflies counted. Mayflies were then sediment gut cleared for 24 hours in a 50 umol EDTA solution (organisms were fed during gut clearing). After gut clearing, mayflies were dried at 60°C for 72 hours, weighed, and placed in cryovials. Endpoints included percent survival and growth. Growth was determined as final wet weight minus the initial wet weight.

2.4.4 Tubifex tubifex chronic (28-day) reproduction and adult survival test

The *T. Tubifex* test was only used to evaluate the spiked Long Point sediment. This test was conducted for 28 days using 4 sexually mature worms per beaker. Prior to the introduction of the worms, 40 mg of crushed Nutrafin® fish flakes were mixed into the sediment and the sediment allowed to settle. On day 28, the contents of each beaker were sequentially rinsed through 500-µm and 250-µm sieves. Using a microscope, the number of surviving adults, full cocoons, empty cocoons, and large immature worms from the 500-µm and the numbers of small immature worms from the 250-µm sieve were counted. Adult worms were gut cleared in a 50 umol EDTA solution for 24 hours and weighed. Worms were not fed during gut clearing but excrement was removed periodically to minimize coprophagy. Worms were then dried at 60°C for 72 hours, reweighed and placed in cryovials. Endpoints included percent survival of adult worms, the total number of cocoons produced per adult, the percent of cocoons that hatched, and total number of young produced per adult.

2.5 Calculation of Critical Concentrations

2.5.1 Critical concentrations based on mortality

Mortality rate was calculated from the test survival as,

$$m = -\mathrm{Ln}(S)/t \qquad (1)$$

where m is the mortality rate per day as a function of the negative natural logarithm of survival (S) per time (t).

The mechanistically based mortality saturation model, as outlined by Norwood *et al.* (2007), was used to define the relationship between exposure concentration (i.e. Zn sediment, water, or body concentrations) and mortality, in order to calculate LC50s;

$$m = m' + (\ln(2)/t)\,[C_{ex}(\mathrm{LC50}^{-1} + K_{ex}''^{-1})\,(1 + C_{ex}\,K_{ex}''^{-1})^{-1}]^{nex} \qquad (2)$$

where m' is the control mortality, K_{ex}'' is exposure concentration (ex), sediment, water or background corrected body concentration, when metal-induced mortality is half of the maximum, C_{ex} is the measured Zn sediment, water or background corrected body concentration, t is the exposure time corresponding to the LC50, the lethal sediment, water or background corrected body concentration at 50% survival and n_{ex} is the exponent.

Mortality rate data were 4th root transformed prior to statistical analyses to normalize the data and then fit to the saturation equation (2) to relate mortality to exposure concentration or tissue concentration. The 4th root transformation produced more uniform variance than log or square root transformations. All models were fit using non-linear regressions in Systat 10 (SPSS Inc.) to estimate each variable (m', LC50, K_{ex}, n_{ex}). In some cases, the estimated value of n_{ex} in equations 2 were extremely large and could not be determined accurately. If estimates of n_{ex} >100, it was set to 100.

LC20s and LC10s were determined as:

$$\mathrm{LC20} = [(\mathrm{LC50}^{-1} + K_{ex}''^{-1})\,(\ln(5/4)\,\ln(2)^{-1})^{1/nex} - K_{ex}''^{-1}]^{-1} \qquad (3)$$

and

$$\mathrm{LC10} = [(\mathrm{LC50}_X^{-1} + K_{ex}''^{-1})\,(\ln(10/9)\,\ln(2)^{-1})^{1/nex} - K_{ex}''^{-1}]^{-1} \qquad (4)$$

where LC20 and LC10 were the lethal sediment, water or background corrected body concentrations at 80% and 90% survival respectively.

2.5.2 Critical concentrations based on growth (wet or dry weight)

The impact of Zn on growth, expressed as final body size W (final wet or dry weight after 10, 21 or 28 days depending on the organism) was evaluated with a general growth model

$$W = W'\,(1 + aC^{n})^{-1} \qquad (5)$$

where W' is the control weight, C is the measured Zn sediment, water or background corrected body concentration, and a and n are constants (Borgmann *et al.*, 1998). Growth data were square root

transformed prior to statistical analyses to normalize the data and equalize variances. This produced more uniform variances than log and 4th root transformation of the data. Growth wet or dry weight was fit to equation (5) using Systat 10, non-linear regression to estimate a and n.

EC50, EC20 and EC10 (sediment, water or background corrected body concentrations resulting in 50%, 20% and 10% reduction in growth) were determined as:

$$EC50 = (1/a)^{1/n} \tag{6}$$

$$EC20 = (1/4/a)^{1/n} \tag{7}$$

$$EC10 = (1/9/a)^{1/n} \tag{8}$$

where a and n were determined from equation (5) based on $(W \bullet W'^{-1} = 0.75 = (1 + aC^{n})^{-1}$ (Borgmann *et al.*, 1998).

3 RESULTS

3.1 Sediment Composition and Metal Concentrations

Three sediments of various compositions were selected in an effort to obtain a wide range of potential zinc binding capacity. The sediment from Long Point was collected in a marsh and had the highest organic and sand content but had the lowest amount of fine clay particles (Table 1). The other Lake Erie sediment (LE303) was collected close to the tip of Long Point peninsula in an area significantly affected by major currents in Lake Erie. This site had low organic carbon and sand content but was dominated by silt and had a fair amount of clay. Lake Restoule was selected as a representative of the "shield" region of Ontario, which is dominated by soft water lakes. Lake Restoule sediment had a high organic content and also had the highest content of the fine clay material (Table 1). Sediments with a higher percentage of fines have a higher binding surface area and along with high TOC, the Zn binding capacity may potentially increase with these types of sediments. Acid volatile sulfide and simultaneously extracted metal analyses were not available, which may have provided additional information on metal bioavailability.

Table 1: Sediment Composition

		LE303	Long Point	Restoule
% Clay	0.1-2.63 um	19	6	52
% Silt	2.63-62 um	75	76	48
% Sand	62-1000 um	6	18	0.2
%Sand,very coarse	1000-2000um	0	0	0
Total Organic Carbon	$mg \bullet g^{-1}$ dry	5	110	93
Total Kjeldahl Nitrogen	$mg \bullet g^{-1}$ dry	0.5	6.3	6.6
Total Phosphorus	$mg \bullet g^{-1}$ dry	0.72	0.88	1.93
Total Solids (L.O.I.)	$mg \bullet g^{-1}$ dry	15	200	314

Table 2: Measured metal concentrations in sediment ($ug \bullet g^{-1}$ dry wt)

	LE 303	Long Point	Restoule
Aluminum	10741	8044	24500
Arsenic	4.6	2.7	0.1
Barium	49	69	388
Beryllium	0.5	1.0	1.5
Cadmium	0.5	0.4	4.4
Calcium	87926	78222	3970
Chromium	17	12	44
Cobalt	7	5	31
Copper	19	16	45
Iron	17370	13000	55700
Lead	9.6	12.6	127.0
Magnesium	17667	9256	3480
Manganese	637	518	5790
Mercury	0.01	0.04	0.25
Molybdenum	0.6	1.0	3.0
Nickel	18	13	50
Strontium	126	137	51
Titanium	392	204	567
Vanadium	28	17	75
Zinc	48	55	339

Background levels of the various elements, measured in the test sediment before the sediments were manipulated, are provided in Table 2. Restoule sediment had the highest background concentrations

of most of the elements measured except strontium, which was highest in Long Point, and arsenic, magnesium and calcium, which were highest in LE303 (Table 2, Figure 1). Overall, Hg, Cd, Pb, Cr, Ni, Cu, Mn and Zn exceeded provincial LEL or federal guidelines ISQG (MOE, 1993; CCME, 1999) in Restoule sediment; Ni, Mn, and Zn guidelines were exceeded in the LE303 sediment; and the Mn guideline was exceeded in the Long Point sediment. Background levels of metals in shield lakes, especial in the "nickel belt region" such as Lake Restoule, can be high due to the natural high concentrations of metals in the bedrock of the region.

Zinc concentrations in the sediments after sediment spiking are provided in Table 3. The nominal 0 ug Zn•g^{-1} dry wt. measured Zn concentrations (Table 3) were lower after the spiking procedure by a factor of 1.8, 1.4 & 1.3 in LE303, Long Point and Restoule sediments, respectively, compared to pre-spiking Zn concentrations (Table 2). A number of other metal concentrations may have also been reduced by the spiking procedure; however, the other metal concentrations in the sediments were not measured after the spiking procedure.

Although the background Zn concentrations in the sediments were reduced by the spiking procedure, a good Zn concentration range was produced (Table 3, Figure 2). Absorption of Zn to the LE303 and the Long Point sediments was very similar; however, a greater absorption of Zn to the Restoule sediments occurred (Figure 2). This was expected since Restoule sediment had a high organic content and a much higher percentage of clay.

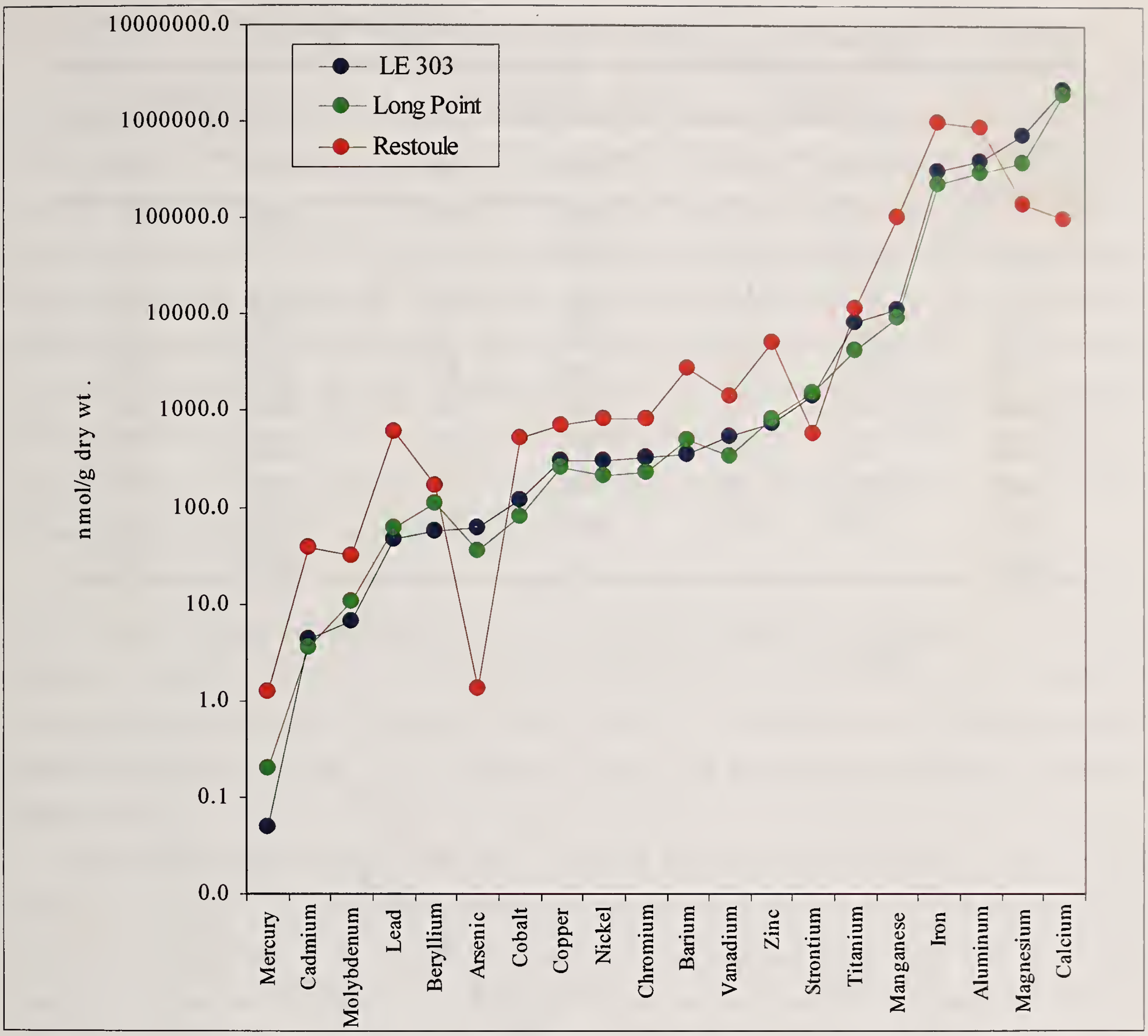


Figure 1 Background concentrations (nmol•g^{-1} dry wt.) of 21 elements in LE303, Long Point and Restoule sediments prior to spiking procedures.

TABLE 3: Measured Zinc concentrations in sediment (ug•g^{-1} dry wt)

Nominal (ul stock)	LE 303		Long Point		Restoule	
	Mean	±Std	Mean	±Std	Mean	±Std
0	26	1.9	38	3.4	253	31
180	152	14	129	4.3		
320	203	4.4	186	5.0	901	25
560	279	60	330	29	1228	63
1000	601	24	488	4.7	1904	48
1800	873	105	869	45	3225	203
3200	1821	260	1597	45	5825	848
5600	2812	178	2725	479	9181	930
10000	4049	1013	4854	2691	13323	37
18000					22163	615

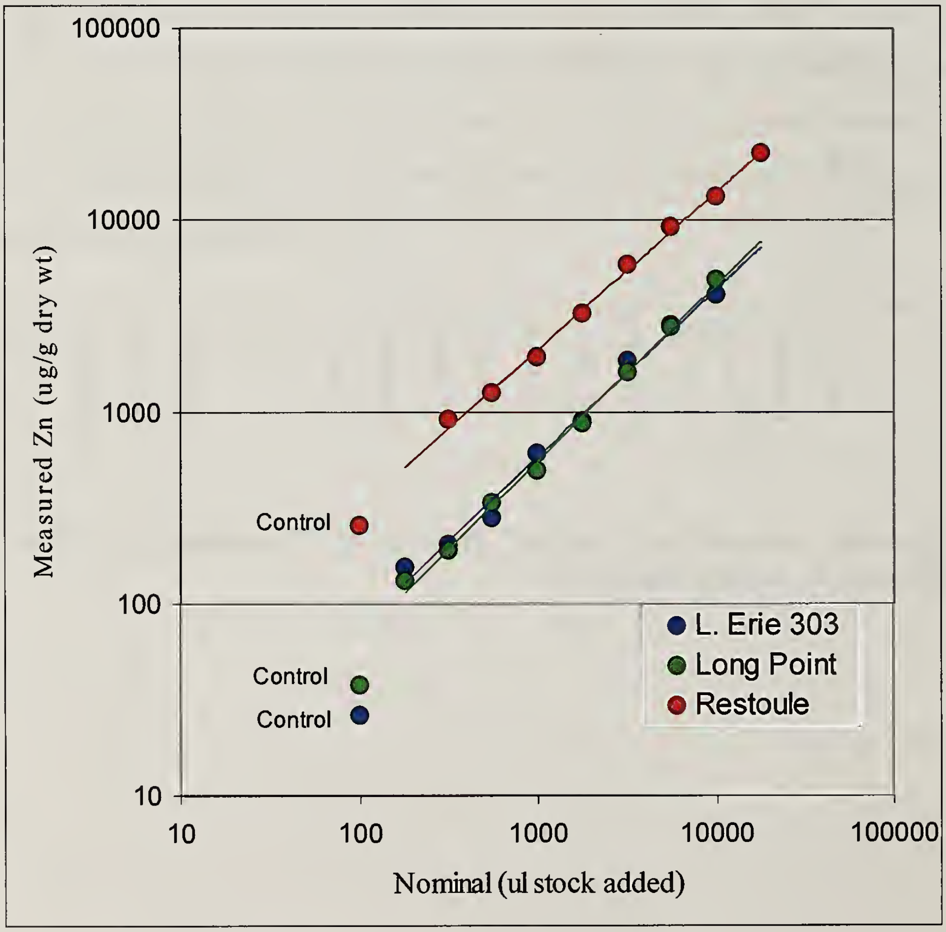


Figure 2 Measured Zn concentrations in the Zn-spiked treatments of sediments collected from; Lake Erie, station 303 (LE303) just off of the Long Point peninsula; a marsh at the base of Long Point peninsula (Long Point); and from Lake Restoule near North Bay Ontario, in the Precambrian shield region (Restoule).

3.2 Overlay Water Characteristics

Major ion and carbon concentrations in the source water (DeChlor) for culture and bioassays was very stable during the testing periods from December 2005 through to April 2007 (mean±Std mg•L^{-1} and sample size (n): dissolved organic carbon (DOC) 0.72± 0.262 (18), dissolved inorganic carbon (DIC) 18.5±1.20 (20), Ca 35.1±1.26 (19), Mg 8.83±0.348 (19), Na 14.4±1.44 (19), K 1.67±0.123 (16), Cl 26.5±1.99 (20) and SO_4 37.8±4.70 (20)). A summary of water quality measurements (pH, total ammonia, dissolved oxygen and conductivity), measured on day 0 and on the last day of the exposure period, is provided in Appendices 1 to 4. Briefly, pH levels in all tests and treatments ranged from 7.6 to 8.6 except in *H. azteca* tests with Lake Restoule sediments and soft, 10% DeChlor water (Appendix 1). Restoule sediments with soft water were tested in four water:sediment ratios (67:1, 200:1, 500:1 and 1000:1) in which the mean pH levels ranged from 6.1 to 8.1 with only one extreme pH reading of 5.4 in a control sediment which had acceptable amphipod survival.

Total ammonia concentrations were negligible in most tests and treatments (Appendix 2). However, total ammonia concentrations did rise in some tests with LE303 sediment, but only at the nominal Zn concentrations greater than 1800 ug•g^{-1} in tests with *C. riparius*. These ammonia levels were not present initially, but only by the end of the exposure. It was possible that the ammonia levels increased in these treatments due to the breakdown of excess food that was not consumed due to high Zn-induced mortality.

Oxygen levels remained within a 1 mg•L^{-1} range of the saturation concentrations of 8.11 to 8.38 mg•L^{-1} for 25°C and 23°C, respectively, for all tests (Appendix 3). One measurement was low at 4.6 mg•L^{-1} but still acceptable as it was greater than 50% of the saturated level. Conductively was also measured in all tests and treatments as an indicator of total ion concentrations and confirmed that with increased Zn concentrations there was an increase in conductivity (Appendix 4). As well, the mean conductivity was 3 to 6 times lower in soft overlay water from the 67:1 through to the 1000:1 water to sediment ratio treatments respectively.

The background Zn retained in the three control sediments, spiked at the nominal level of 0 ug•g-1 dry wt. (Table 3), were quite different. Site LE303 was the lowest followed by Long Point and Restoule contained the highest Zn concentration. However, the amount of Zn that is released from test sediment to the overlay water may be important as it may become bioavailable, especially if the route of exposure of Zn to an organism is dominated by the waterborne pathway as opposed to the ingestion pathway. There may be a number of factors that control the release of Zn from sediment, such as the ratio of water to sediment in the test, binding or complexing components in the sediment as well as factors in the overlying water and possibly duration of an experiment. The four toxicity tests employed in this study were different in a number test conditions such as duration, water to sediment ratio, feeding regime, overlay

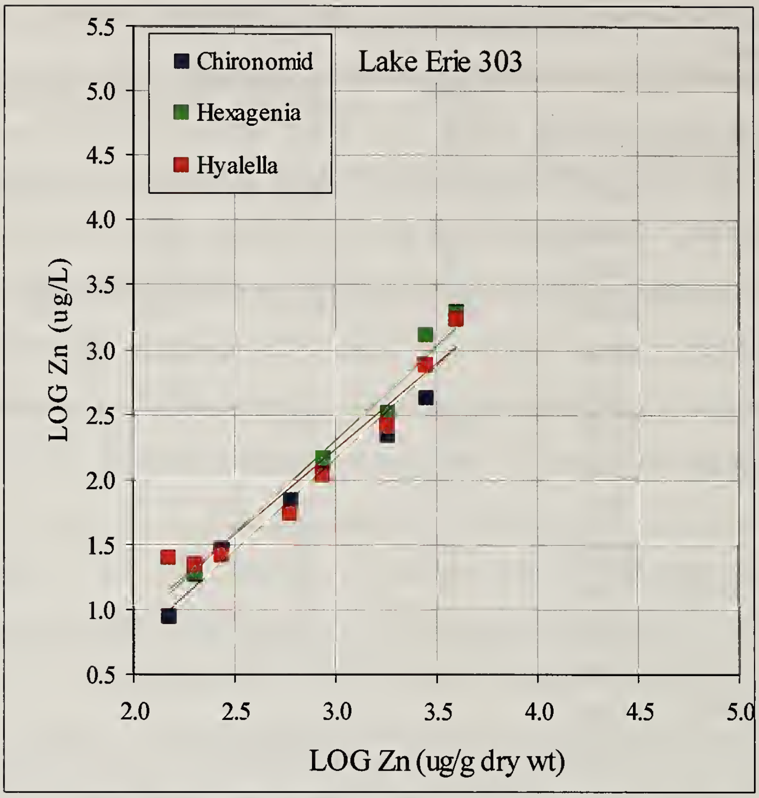
water type (hard & soft in the *H. azteca*, Restoule tests), animal behaviour as well as different sediment types. The impact of these various conditions can be clearly seen in the release of Zn from the control sediment (0 Zn spike) to the overlay water (Table 4). A higher Zn concentration in the sediment did not always result in a higher Zn concentration in the overlay water. Overlay water Zn concentrations were 11% and 68% lower in the *H. azteca* and *C. riparius* tests respectively, in LE303 sediment exposures compared to Long Point sediment exposures. Higher Zn concentrations did occur in the overlay water in the *H. azteca* test with Restoule sediment (67:1 ratio), however, the Zn overlay water concentration was only 2 and 2.3 times higher in comparison to the LE303 and the Long Point overlay water concentrations, respectively, even thought the Restoule sediment concentration was 9.7 and 6.7 times higher, respectively (Table 4).

In Restoule sediment exposures, Zn release from the control sediment was considerably lower in hard water (100% DeChlor) than in soft water (10% DeChlor) such that Zn water concentrations were 76%, 36%, 33% and 6% lower in the 67:1, 200:1, 500:1 and 1000:1 ratios respectively (Table 4). Therefore, a decrease in hardness corresponded to an increase in Zn release from the sediment and higher Zn concentrations in the overlay water. The water to sediment ratio also had an impact on the release of Zn from the sediment to the overlay water. As the water to sediment ratio increased from 200:1 to 1000:1 there was a corresponding increase in Zn overlay water concentration in the *H. azteca* tests in both the hard and soft overlay waters (Table 4). The concentrations observed in the 67:1 ratio test was not included in this comparison since this test was conducted one year earlier than the tests with the 200:1, 500:1 and 1000:1 ratios, and therefore the sediment had not aged as long. The additional aging reduced the release Zn.

Table 4: Measured Zn water concentrations ($ug \cdot L^{-1}$) in control treatments. Ratio is overlay water volume divided by sediment volume.

		LE 303	Long Point	Restoule	Restoule	Restoule	Restoule
Control Sediment Zn ($ug \cdot g^{-1}$ dry wt)		26	38	253	253	253	253
	Ratio	67:1	67:1	67:1	200:1	500:1	1000:1
Hyalella (100%) DeChlor	mean	14	13	30	5	14	27
	Std	8	9	31	1.1	12.7	34.7
	Ratio	67:1	67:1	67:1	200:1	500:1	1000:1
Hyalella (10% DeChlor)	mean			126	13	20	29
	Std			67	11	11	19
	Ratio	10:1	10:1				
Chironomid (100% DeChlor)	mean	11	3.5				
	Std	7	2.2				
	Ratio	10:1	4:1				
Hexagenia (100% DeChlor)	mean	17	38				
	Std	7	8				
	Ratio		10:1				
Tubifex (100% DeChlor)	mean		8				
	Std		1.8				

There was a linear increase in Log Zn concentration in the overlay water of all tests with LE303 spiked sediments (Fig. 3, top panel). As well, the water concentrations were very similar in all tests. A similar trend was observed in tests conducted with Long Point spiked sediments (Figure 3, lower panel), however, water concentrations remained at background levels until the sediment concentrations exceeded 327 $ug \cdot g^{-1}$ dry weight. Zinc water concentrations in the *H. azteca* tests with Restoule sediment also demonstrated the same linear trend as that of LE303, however there were some differences in the different water to sediment ratio treatments as well as differences between hard and soft waters (Figure 4). The greater the water to sediment ratio, the lower the Zn water concentrations and hence the slopes of the linear regressions were very similar (Fig. 4, upper and lower panels), but the y intercept decreased with increasing water:sediment ratio (i.e. the curves shifted down). Overall, the release of Zn from the sediment was very similar for all three sediments, especially when compared using the same test setup (Figure 5). In this case, Zn water concentrations in the *H. azteca* tests, using a 67:1 water to sediment ratio with hard water (100% DeChor), were very similar at the same sediment concentrations. However, Zn concentrations in soft water (10% DeChlor), using a 67:1 water:sediment ratio, were significantly higher at the same sediment concentrations (Figure 5).



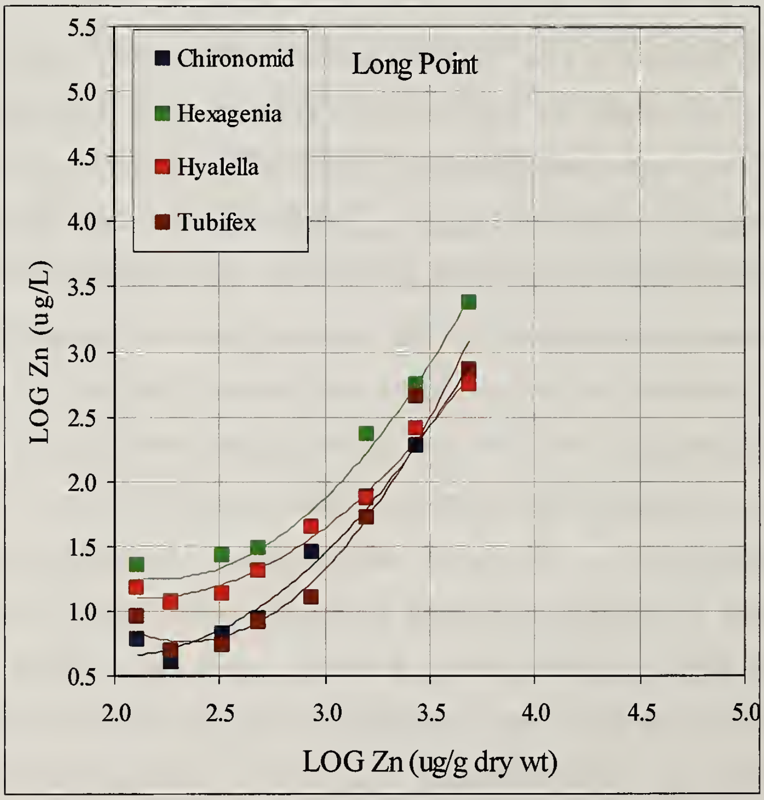


Figure 3 Zinc concentrations in overlay waters exposed to site LE303 (top panel) and Long Point (bottom panel) sediments for all four organisms tested.

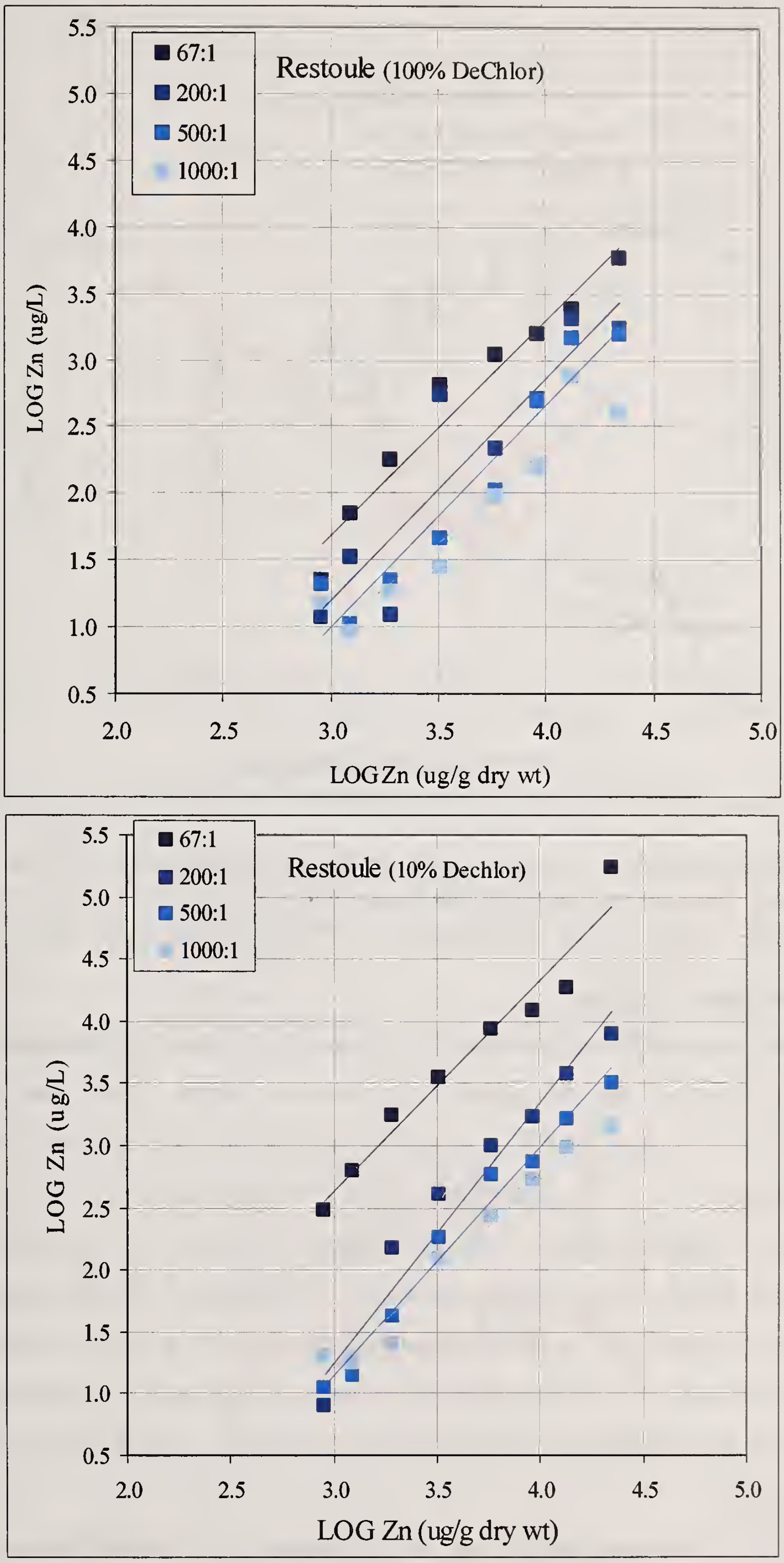


Figure 4 Mean Zn concentration in hard (100% DeChlor - upper panel) and soft (10% DeChlor - lower panel) overlay waters from 28-day *H. azteca* tests with Lake Restoule sediments. Four different ratios of overlay water volume to sediment volumes were used, 67:1, 200:1, 500:1 and 1000:1.

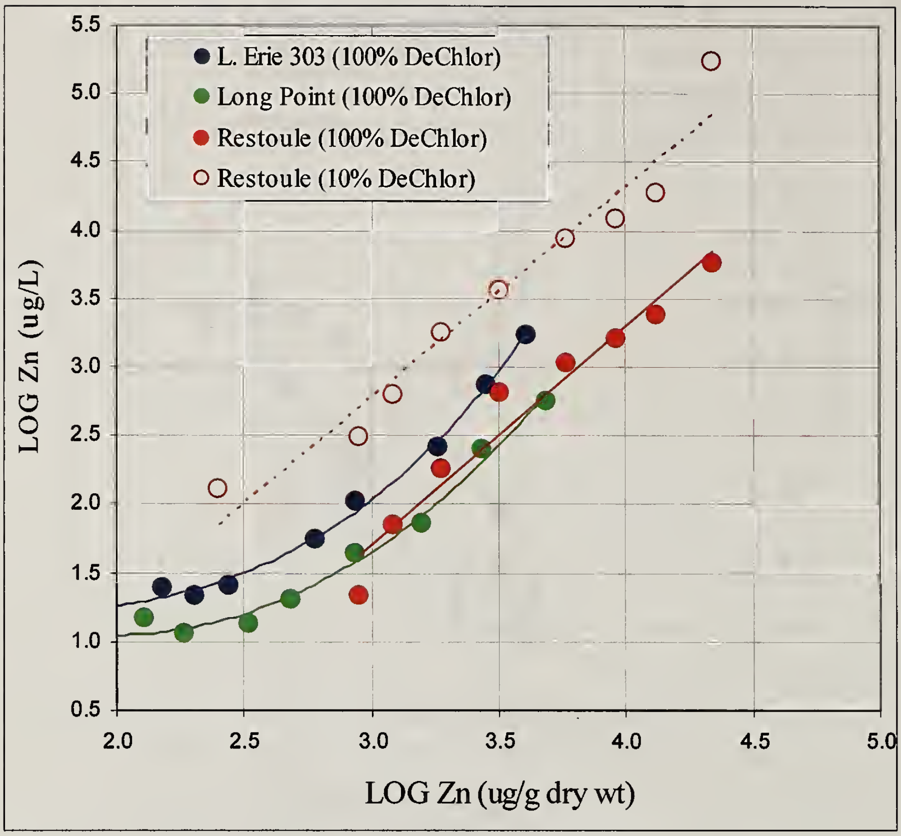


Figure 5: Zinc concentrations in overlay waters from *H. azteca* exposures to Lake Erie, sites 303 and Long Point, and Lake Restoule sediments, all at a water to sediment ratio of 67:1. Overlay waters were 100% DeChlor (hard water) for all sediments and 10% DeChlor (soft water) for Restoule only

3.3 Bioaccumulation of Zinc

T. tubifex body concentrations increased by a factor of 19 above background levels (Fig. 6 & 8, lower panels) when exposed to the spiked Long Point sediment. There was also a fairly good bioaccumulation of Zn in *Hexagenia* spp. by a factor of 10 times the background levels with exposure to LE303 sediments (Figure 6 & 8, top panel). However, with the exposure to the Zn-spiked Long Point sediments, the highest *Hexagenia* spp. tissue concentrations were only 1.8 times higher than background levels (Figure 6, lower panel). *C. riparius* bioaccumulation did increase with increasing sediment or water Zn concentrations with both LE303 and Long Point exposures (Fig. 6 & 8) but only by factors of 6.7 and 3.8 respectively. There was very little difference between background (control) and spiked sediment exposed *H. azteca* body concentrations with exposures to LE303, Long Point and Restoule Zn spiked sediments (Figs. 6 to 9).

When tissue Zn concentrations for all the organisms were plotted versus the overlay water concentrations, the relationship was more linear providing a better fit (i.e. Zn water concentration was a better predictor of tissue Zn concentrations) for all organisms in exposures to the LE303 and Long Point sediments (Figure 8, upper and lower panels, respectively).

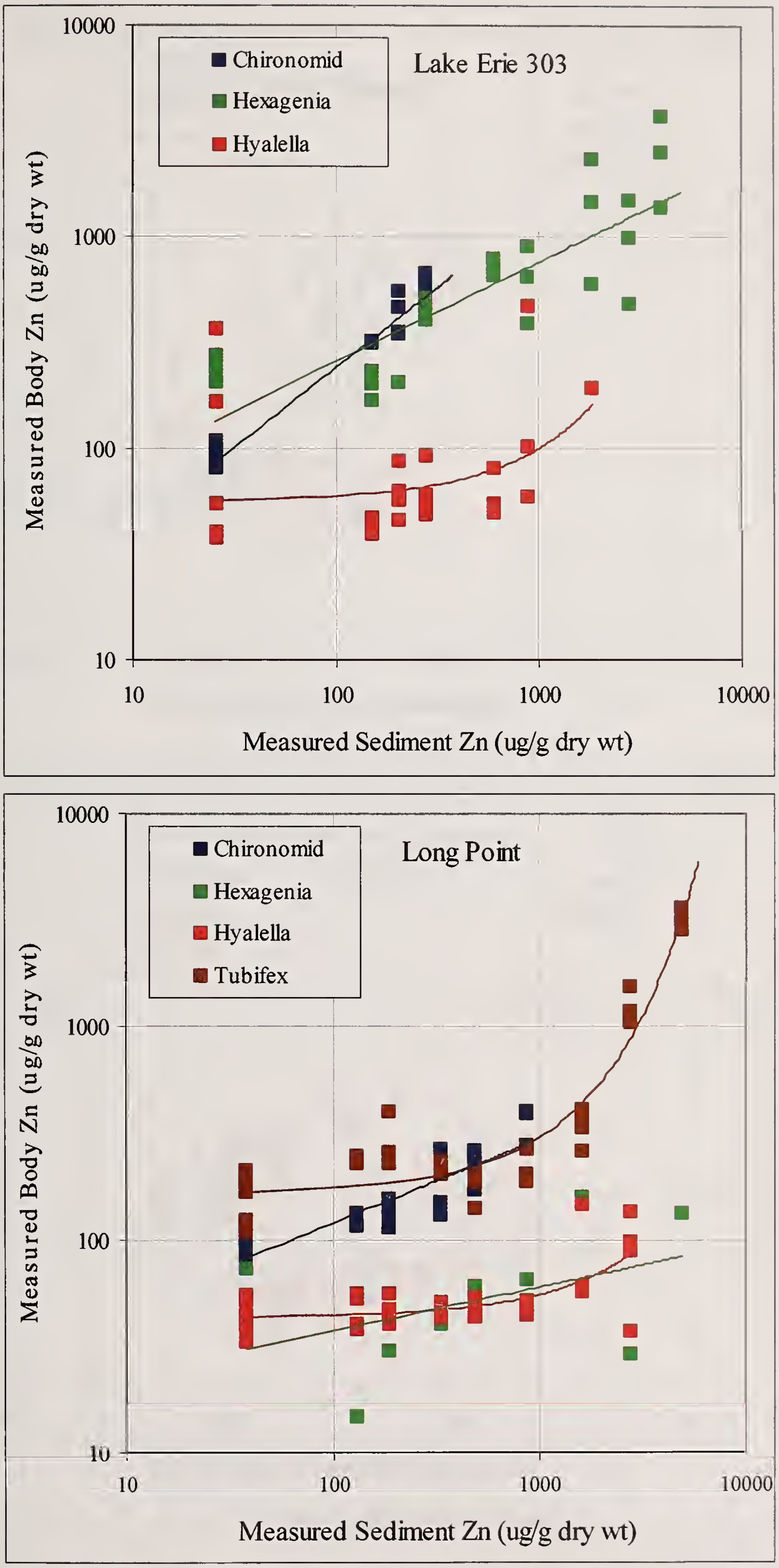


Figure 6 Bioaccumulation in relation to LE303 (upper panel) and Long Point (lower panel) sediment concentrations.

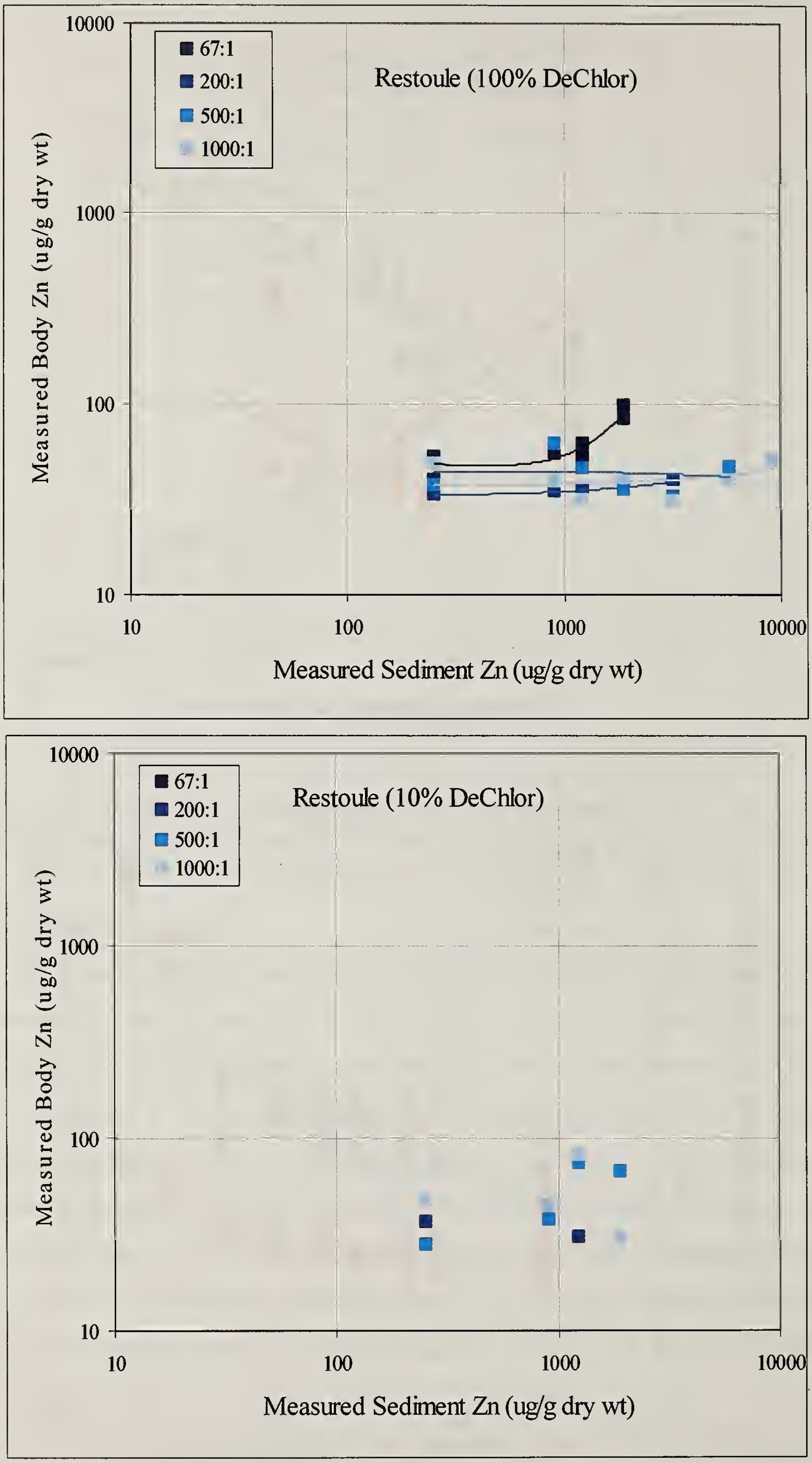


Figure 7 Bioaccumulation in relation to Lake Restoule sediment concentrations with hard (100% DeChlor, upper panel) and soft (10% DeChlor, lower panel) overlay water,

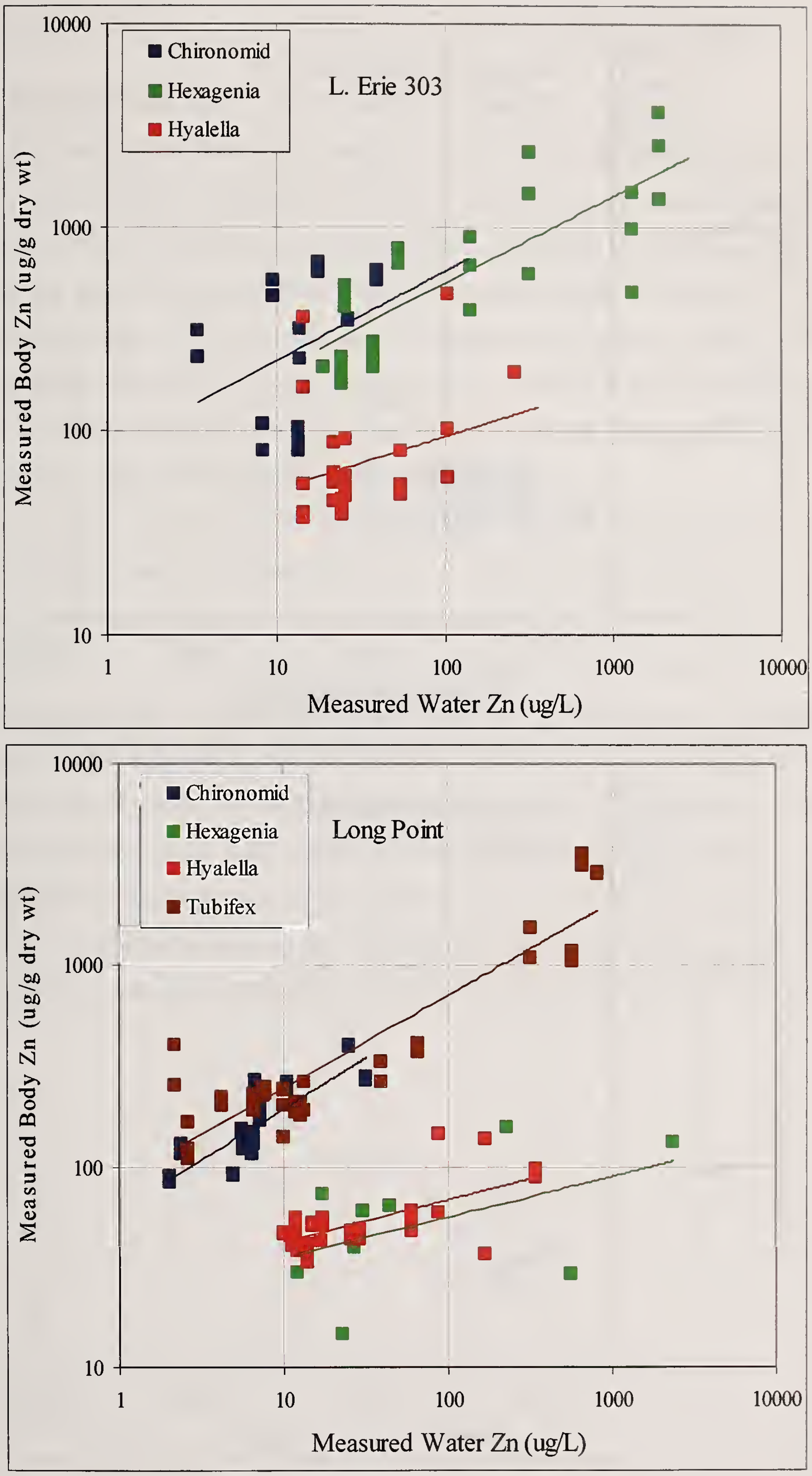


Figure 8 Bioaccumulation in relation to LE303 (upper panel) and Long Point (lower panel) overlay water concentrations.

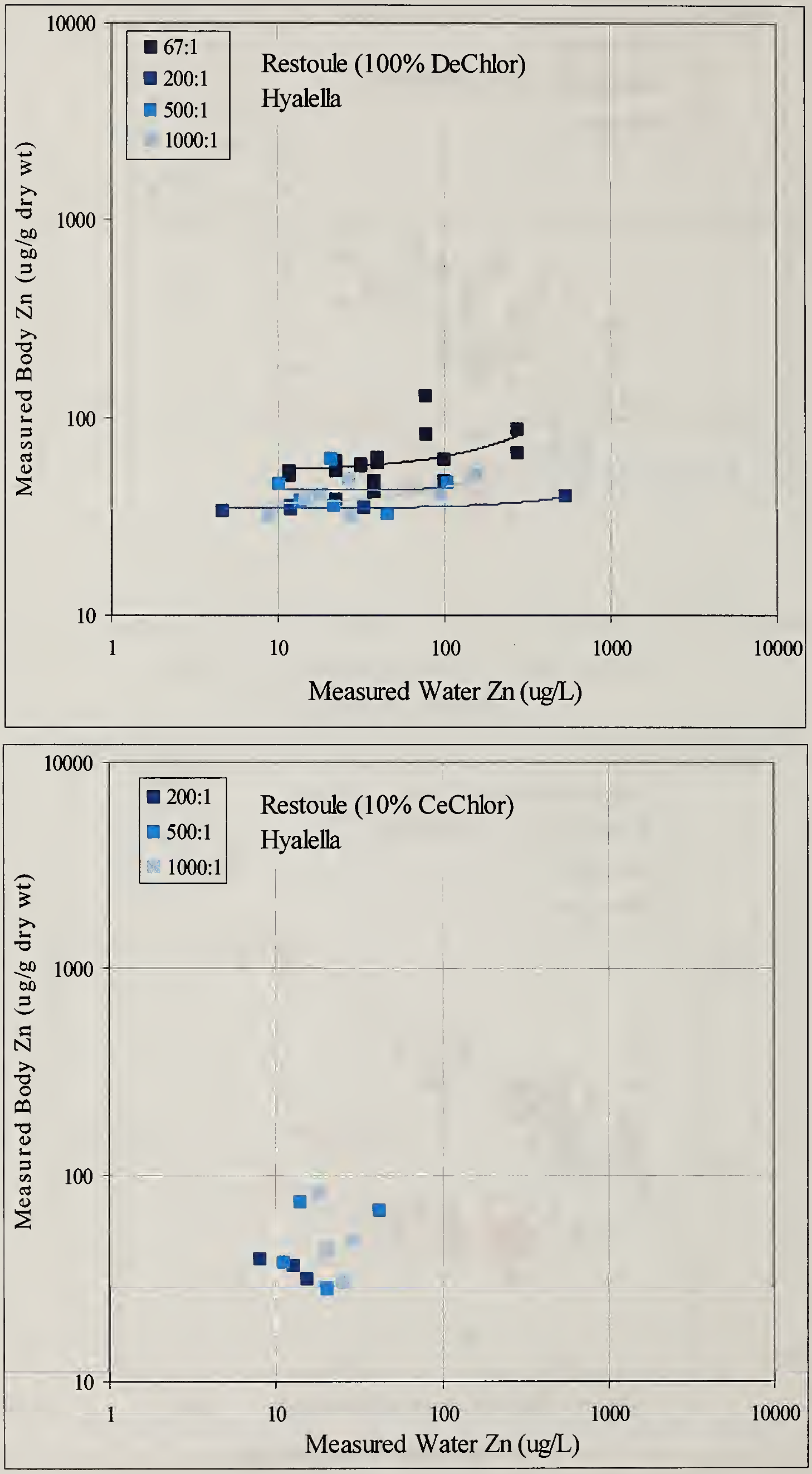


Figure 9 Bioaccumulation in relation to Lake Restoule hard (100% DeChlor, upper panel) and soft (10% DeChlor, lower panel) overlay water Zn concentrations.

3.4 Toxicity

3.4.1 Toxicity Related to Sediment Concentrations

Survival was reduced to zero with exposure to the LE303 sediment by 601 ug Zn•g^{-1} dry wt sediment for *C. riparius*, and by 2810 ug Zn•g^{-1} dry wt sediment for *H. azteca* (Figure 10). *C. riparius* and *H. azteca* followed very similar trends in survival with exposures to increasing Zn concentrations in both LE303 and the Long Point sediments (Figure 11, upper panels); however, *C. riparius* appears slightly more sensitive than *H. azteca*. *H. azteca* demonstrated a hormetic effect in the intermediate exposure concentrations (Figure 11, upper right panel). Survival was not significantly reduced in *Hexagenia* spp. in LE303 (Figure 10) or in Long Point Zn-spiked sediment (not plotted); therefore, critical concentrations could not be calculated for either sediment.

Survival of *H. azteca* exposed to Zn-spiked Restoule sediment with hard (100% DeChlor) overlay water was similar for the water to sediment ratios of 67:1, 200:1, 500:1 and 1000:1 (Figure 11, lower left panel). However, there was more scatter in the data probably due to fewer number of replicates per treatment. In exposures of *H. azteca* with soft (10% DeChlor) overlay water, the curves were shifted to the left indicating greater toxicity (Figure 11, lower right panel), but there were only single replicates in these tests and hence the curves are not well defined. There was no survival in the soft water, 67:1 treatment at any Zn concentration. As well, the pH was low (mean of 7.2, Appendix 1) compared to the 100% DeChlor treatments, mean total ammonia was 0.18 mmol•L^{-1} (Appendix 2) for all the Zn concentrations and the conductivity was about 1/3 of the level of the 100% DeChlor (Appendix 4). This may indicate that the amphipods were at their tolerance limits of ion balance and the lower buffering capacity, in combination with the additional stress of lower pH and the presence of some ammonia was detrimental.

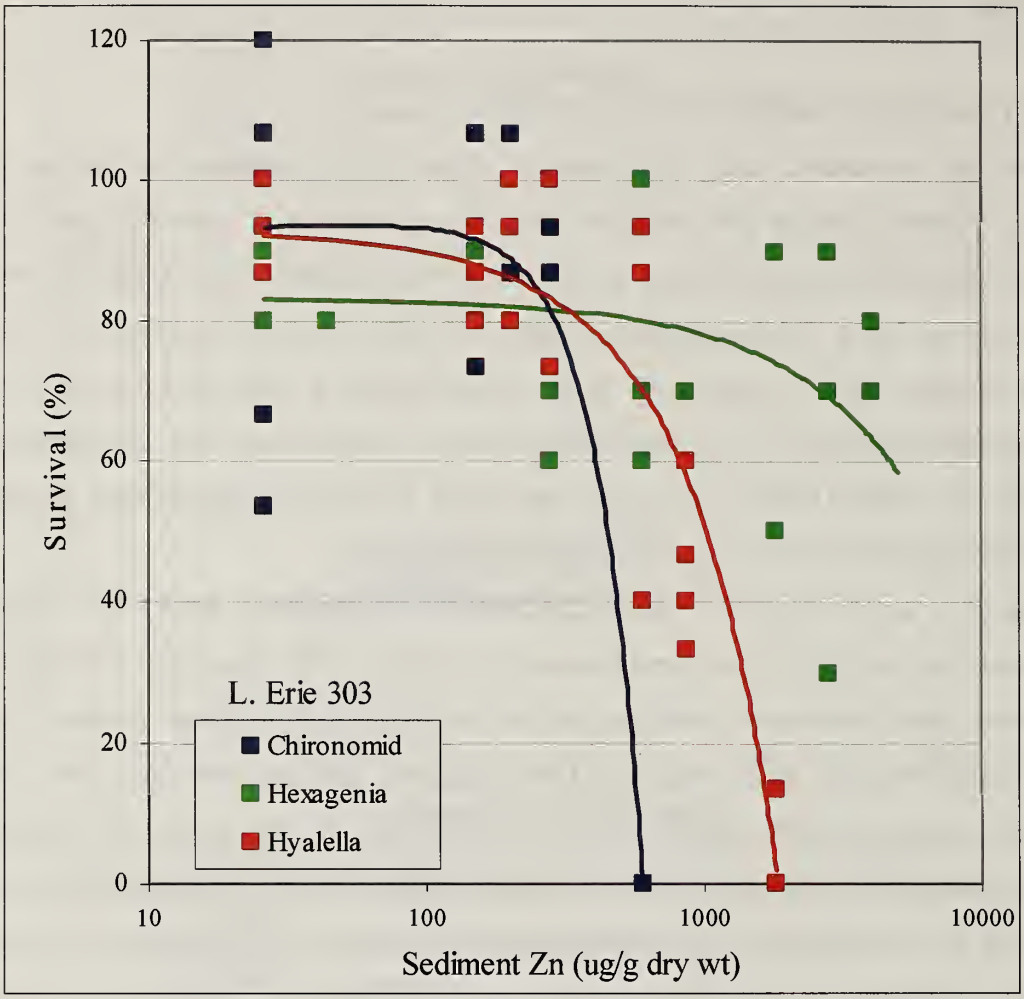


Figure 10 Mean survival of *C. riparius*, *Hexagenia* spp. and *H. azteca* versus measured Zn sediment concentrations.

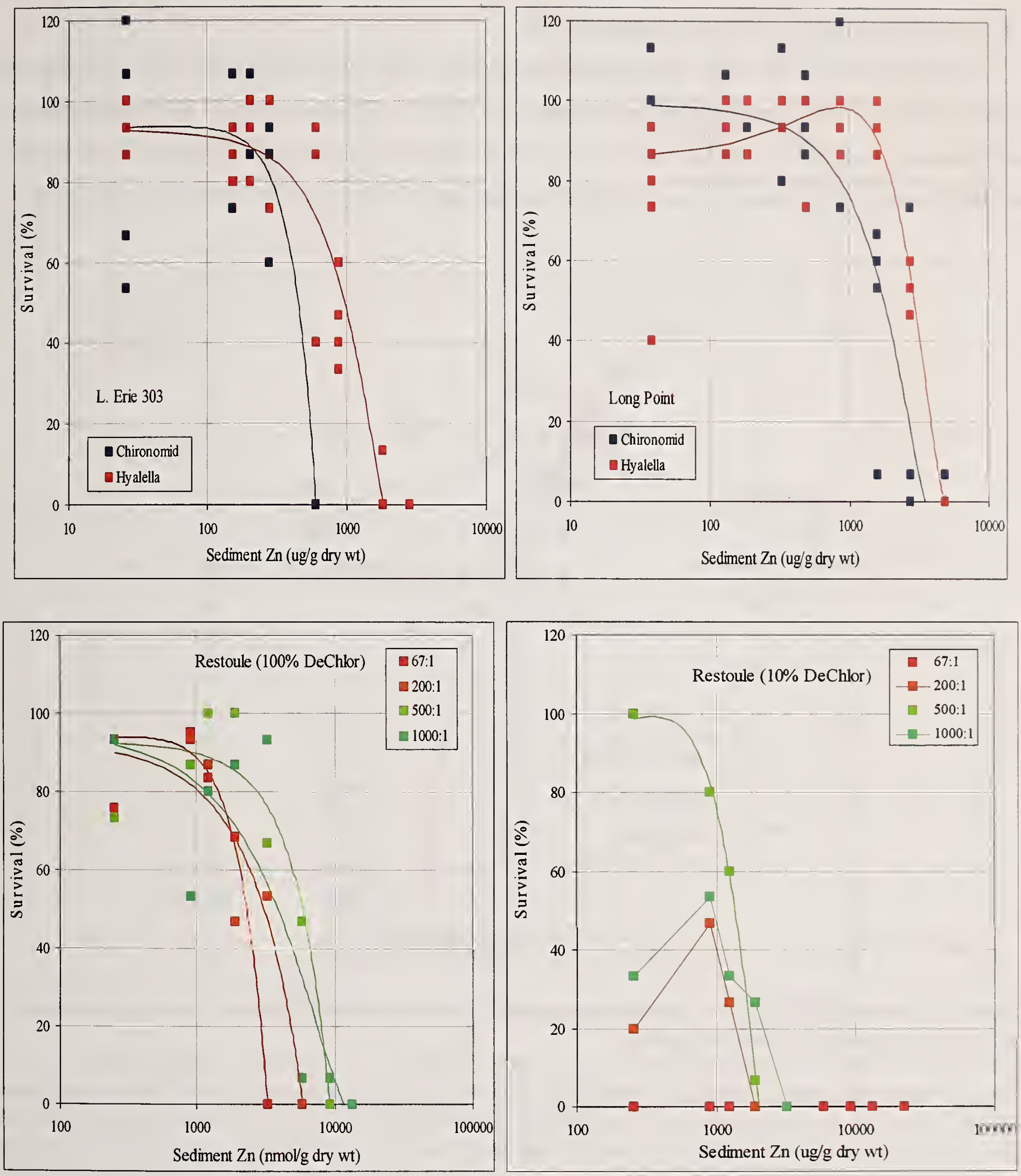


Figure 11 Mean survival of *C. riparius* and *H. azteca* versus measured zinc concentrations in LE303 Long Point and Restoule sediments. Restoule sediments were tested with four different ratios of overlay water volume to sediment volumes (67:1, 200:1, 500:1, 1000:1) and two different water hardness levels (100% (hard) and 10% DeChlor (soft)).

3.4.2 Toxicity Related to Water Concentrations

Relating the survival of the test organisms to overlay water Zn concentrations did not change the toxicity patterns to any significant amount (Figures 12 and 13). Therefore, critical water concentrations could be calculated for: *C. riparius* and *H. azteca* in exposures to Zn-spiked LE303 and Long Point as well as *H. azteca* in exposures to Zn-spiked Restoule sediments in both hard and soft water treatments.

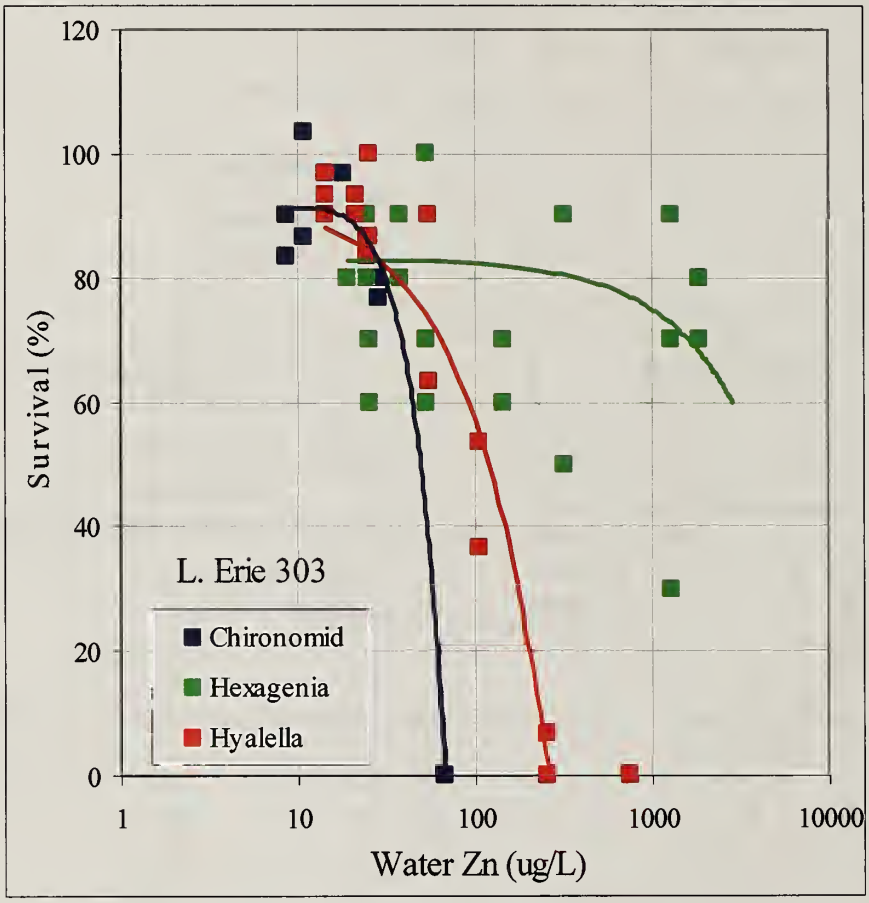


Figure 12 Mean percent survival of test organisms in relation to overlay water zinc concentrations in Lake Erie 303 spiked sediment exposures.

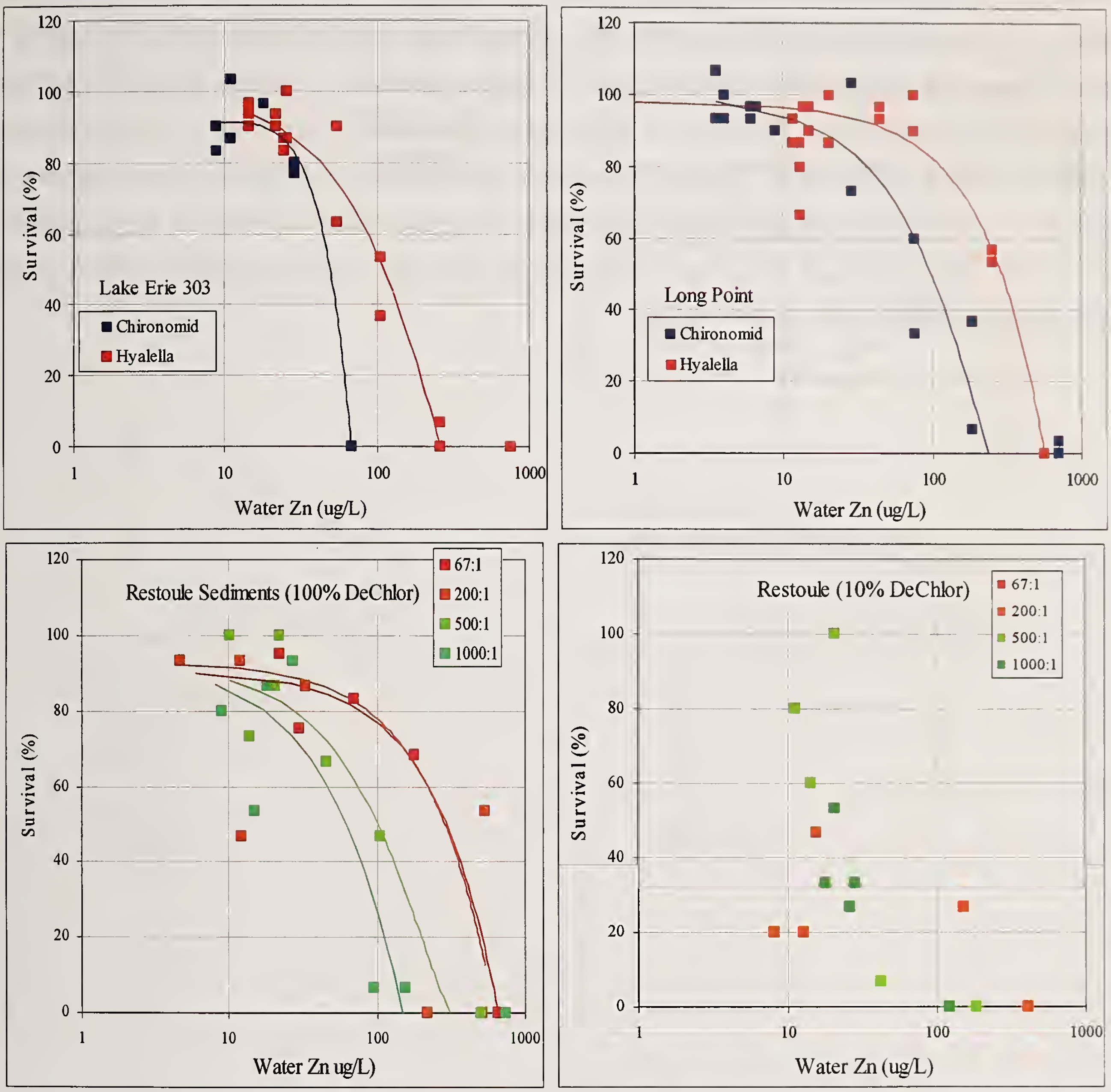


Figure 13 Mean percent survival of *H. azteca* and *C. riparius* in relation to overlay water zinc concentrations in LE303 (upper left panel) and Long Point (upper right panel) spiked sediment exposures and mean percent survival of *H. azteca* in relation to overlay water zinc concentrations in Restoule spiked sediment exposures with hard (lower left panel) and soft (lower right panel) overlay waters and various water: sediment ratios.

3.4.3 Impact on Growth

There was an impact on the growth (based on wet weight) of *C. riparius* and *H. azteca* with exposure to Zn-spiked LE303 sediments (Figure 14, upper left panel) and therefore effect concentrations could be calculated for these two species. Growth (wet or dry wt.) data was not collected for *Hexagenia* spp., hence effect concentrations could not be calculated for this species. There was an impact on the

growth of *C. riparius*, *H. azteca* and *Hexagenia* spp. with exposures to Zn-spiked Long Point sediments (Figure 14, upper right panel) and therefore effect concentrations could be calculated for these three species. A hormetic growth effect was observed for *T. Tubifex* exposed to Zn-spiked Long Point sediment (Figure 14, upper right panel). There was no impact on growth (wet wt.) of *H. azteca* with exposures to Zn-spiked Restoule sediments in either of the hard or soft water tests (Figure 14, lower panels) and therefore no effect concentrations could be calculated. The growth patterns based on dry weight were basically the same as the patterns for wet weight and therefore the corresponding effect concentrations were calculated (data not shown).

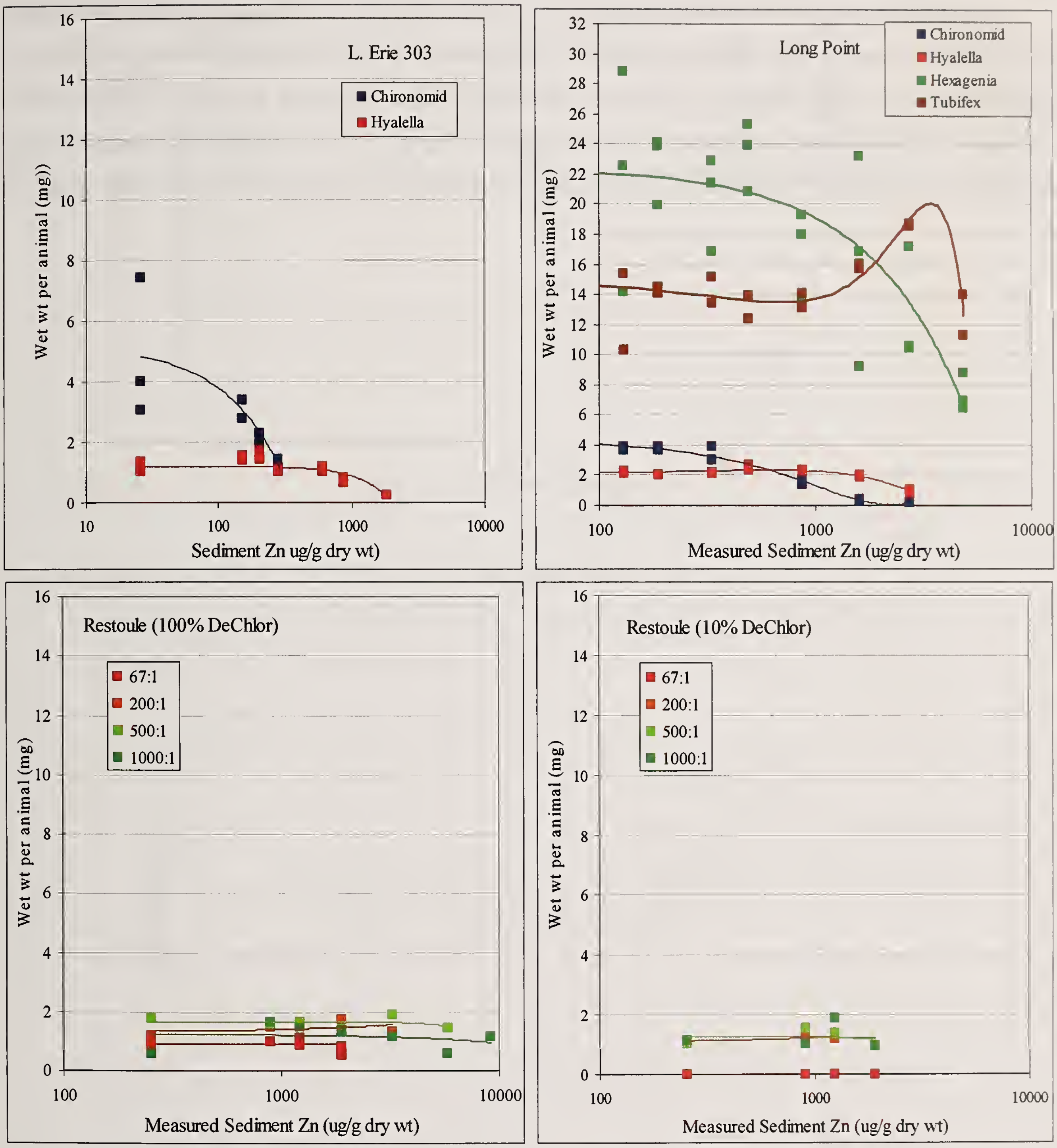


Figure 14 Mean wet weight of *H. azteca and C. riparius* in relation to measured sediment zinc concentrations in Lake Erie 303 (upper left panel) and for all four species exposed to Long Point Zn spiked sediment (upper right panel) and mean wet weight of *H. azteca* in relation to measured sediment zinc concentrations in Restoule sediment exposures with hard (lower left panel) and soft (lower right panel) overlay waters and various water: sediment ratios.

3.4.4 Impact on Reproduction (T. tubifex)

The sharp increase of Zn bioaccumulation in *T. tubifex* (Figure 15, left panel) corresponds directly to a hormetic increase in total offspring (Figure 15 right panel). As bioaccumulation increases there was a sudden decrease in total offspring production. This type of data could not be fit to an effects model; however, at a Zn sediment concentration greater than 2730 ug•g^{-1} dry wt., significant impacts on young production were evident. Therefore, the EC10, EC20 and EC50 was between 2730 and 4860 ug•g^{-1} dry wt.

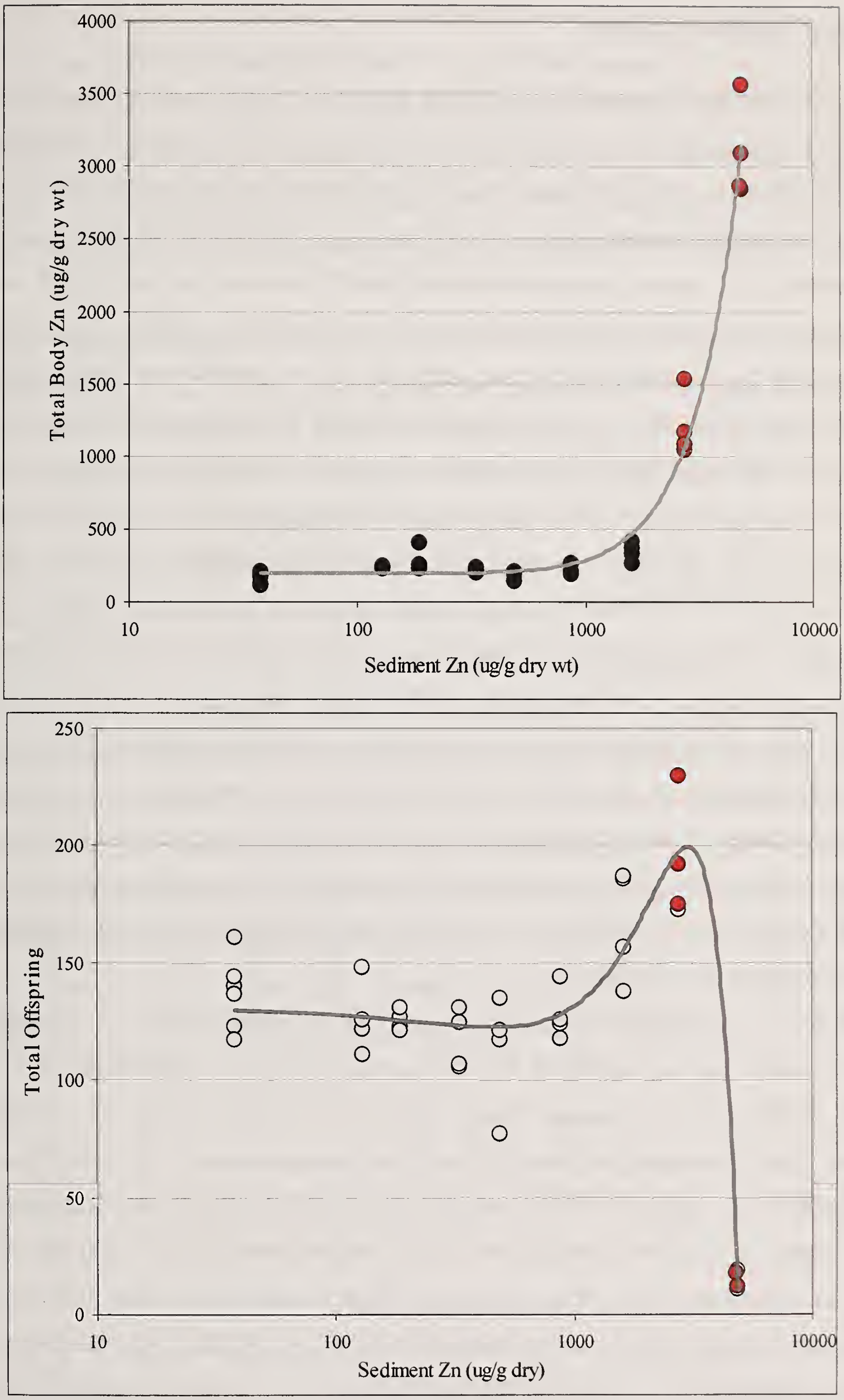


Figure 15 Zinc bioaccumulation (total body concentration) in *T. tubifex* exposed to Zn-spiked Long Point sediment (upper panel). Reproduction (total offspring) produced by *T. tubifex* exposed to Zn-spiked Long point sediment (lower panel).

3.5 Critical Concentrations

Critical lethal sediment concentrations (LC50, LC20 and LC10) were calculated with a saturation mortality model by non-linear regression for all tests where an impact on survival was observed (Table 5). This could not be done for *Hexagenia* spp. since its survival was not affected by either LE303 or Long Point Zn-spiked sediments. As well, there was no impact on survival of *T. tubifex* exposed to Zn-spiked Long Point sediments. *C. riparius* was more sensitive than *H. azteca* to both the LE303 and Long Point, Zn-spiked sediments; *C. riparius* LC50s, LC20s and LC10s were on average two times lower that those for *H. azteca* (Table 5). *H. azteca* was the only organism used to test the Restoule Zn-spiked sediments and the critical lethal sediment Zn concentrations determined with hard overlay water (100% DeChlor) were higher (less toxic) than the LE303 sediments and were lower (more toxic) than the Long Point sediments when looking at tests with the same water to sediment ratio of 67:1 (Table 5). However, when soft overlay water (10% DeChlor) was used with the Restoule Zn-spiked sediments, the toxicity was significantly increased such that there was no *H. azteca* survival at any Zn concentration, including in the controls at water to sediment ratio of 67:1 (Table 5). *H. azteca* survival in Restoule sediments with soft overlay water only occurred when the water to sediment ratio was at 200:1 and higher (Fig. 13, lower right panel) but only critical lethal concentrations could be calculated with the 500:1 test since control survivals were not adequate in the 200:1 and the 1000:1 tests. In summary, *C. riparius* was the most sensitive organism based on survival with an LC10 of 269 ug Zn•g^{-1} dry wt. for LE303 sediment (Table 5). The toxicity ranking of the different sediments, from most toxic to least toxic, based on *H. azteca* was: Restoule (soft water) > LE303 (hard water) > Restoule (hard water) > Long Point (hard water), tested at the 67:1 water to sediment ratio (Table 5).

Growth was a more sensitive endpoint than survival for *C. riparius* and *H. azteca* with exposures to both the LE303 and Long Point sediment. For *C. riparius*, the effect concentrations (EC50s, EC20s and EC10s) were 64.2% lower on average (range: 49.5 to 72.7% lower) than the corresponding lethal concentrations (Table 6 compared to Table 5). The effect concentrations for *H. azteca* were 22.1% lower on average (range: 7.5% higher to 40.0% lower) than the corresponding lethal concentrations (Table 6 compared to Table 5). However, growth was a more variable endpoint and thus the R^2 values were generally not as significant as those seen for survival (Table 6 compared to Table 5). For example, the R^2 value for the growth model with *C. riparius* exposed to LE303 was 0.528 (Table 6) where as it was 0.712 for the lethality model (Table 5). However, the growth model for *C. riparius* exposed to Zn-spiked Long Point sediment was the exception and had a good relationship to Zn sediment concentration with an R^2 of 0.853 (Table 6) compared to the lethality model at 0.739 (Table 5). A growth model could not be calculated for *H. azteca* exposed to Zn-spiked Restoule sediments.

Hexagenia spp. effect concentrations were calculated for the Zn-spiked Long Point sediments and were found to be the higher (less sensitive) than *C. riparius* and similar to *H. azteca* (Table 6). Both *Hexagenia* spp. and *H. azteca* EC values were variable with large 95% confidence limits in comparison to *C. riparius*. There was no impact on *T. tubifex* growth by Long Point, Zn-spiked sediments. The growth models based on dry weights generally produced similar effect concentrations to that of the growth models based on wet weights such that the value usually fell within the 95% confidence interval (Table 5 and 6).

Critical concentrations, both lethality and effect, were also calculated based on the overlay water concentrations (Tables 8, 9 and 10) and generally resulted in similar R^2s, the same ranking of organism sensitivities and the same ranking of the different sediment toxicities. The critical water concentrations were calculated in order to compare the consistency to that of the critical sediment values. The critical water concentrations for *H. azteca* produced the same ranking of the sediments (i.e. toxicity: LE303 > Long Point > Restoule) using hard water at the 67:1 ratio, and the variability from low to high critical values were very similar (Tables 5 versus 8 and Table 6 versus 9).

There were no strong relationships between tissue zinc concentrations and survival (Table 11) and hence critical lethal tissue concentrations could not be calculated for *C. riparius*, *Hexagenia* spp. and *T. tubifex*. The relationship between *H. azteca* tissue concentrations and lethality was weak (R^2 values were <0.35). However, bioaccumulation could be linked to growth impairment based on wet weight in *C. riparius* exposures to LE303 and Long Point Zn-spiked sediments (Table 12). Also, effect concentrations (growth impairment) based on tissue concentration could be determined for *C. riparius*, *Hexagenia* spp. and *H. azteca* exposures to Long Point Zn-spiked sediments, however, only the *C. riparius* growth model had a good R^2 value of 0.844. Even fewer growth models based on dry weight could be fit to bioaccumulation data (Table 13) and these results produced similar results as those fit with wet weight (Table 12).

Table 5. Critical Zn sediment concentrations (ug•g^{-1} dry wt) at 50, 20 and 10 percent mortality with 95% confidence limits, in exposures of the four test organisms to different sediments and overlay waters. Corrected R^2 indicates the relative predictive power of the mortality model.

			Ratio	Zn μg•g^{-1} dry wt			
Sediment	Organism	Overlay Water	Water:Sediment	LC50 (95% C.I.)	LC20 (95% C.I.)	LC10 (95% C.I.)	R^2 Corr
LE303	*Chironomid (10-day)*	DeChlor (100%)	10:1	380 (291- 469)	304 (214 - 394)	269 (167 - 357)	0.712
LE303	*Hexagenia (21-day)*	DeChlor (100%)	10:1	N.I.	N.I.	N.I.	NA
LE303	*Hyalella (28-day)*	DeChlor (100%)	67:1	882 (706 - 1066)	628 (474 - 778)	526 (385 - 667)	0.784
Long Point	*Chironomid (10-day)*	DeChlor (100%)	10:1	1556 (1151 - 1961)	1039 (719 - 1360)	850 (564 - 1137)	0.739
Long Point	*Hexagenia (21-day)*	DeChlor (100%)	4:1	N.I.	N.I.	N.I.	NA
Long Point	*Hyalella (28-day)*	DeChlor (100%)	67:1	3014 (2432 - 3595)	2399 (1791 - 3001)	2111 (1510 - 2713)	0.681
Long Point	*Tubifex (28-day)*	DeChlor (100%)	10:1	N.I.	N.I.	N.I.	NA
Restoule	*Hyalella (28-day)*	DeChlor (100%)	67:1	2294 (1843 - 2746)	1909 (1392 - 2419)	1713 (1183 - 2242)	0.707
Restoule	*Hyalella (28-day)*	DeChlor (100%)	200:1	2393 (974 - 3818)	1608 (429 - 2791)	1320 (256 - 2386)	0.902
Restoule	*Hyalella (28-day)*	DeChlor (100%)	500:1	4831 (922 - 8760)	3550 (-163 - 7256)	3020 (-520 - 6537)	0.717
Restoule	*Hyalella (28-day)*	DeChlor (100%)	1000:1	4968 (1235 - 850)	3569 (273 - 6864)	3001 (-2.48 - 6008)	0.796
Restoule	*Hyalella (28-day)*	DeChlor (10%)	67:1	---------- C.N.C. (100% Mortality in all treatments) ---------			
Restoule	*Hyalella (28-day)*	DeChlor (10%)	200:1	---------------- C.N.C. (Poor Control Survival) ----------------			
Restoule	*Hyalella (28-day)*	DeChlor (10%)	500:1	1249 (804 - 1693)	896 (506 - 1288)	758 (394 - 1118)	0.982
Restoule	*Hyalella (28-day)*	DeChlor (10%)	1000:1	---------------- C.N.C. (Poor Control Survival) ----------------			

N.I. - No Impact
C.N.C. - Could Not Calculate

Table 6. Critical Zn sediment concentrations (ug•g^{-1} dry wt) at 50, 20 and 10 percent reduction in growth (**wet wight**) with 95% confidence limits in exposures of the four test organisms to different sediments and overlay waters. Corrected R^2 indicates the relative predictive power of the growth model.

				Zn µg•g^{-1} dry wt			
Sediment	Organism	Overlay Water	Water:Sediment	EC50	EC20	EC10	R^2 Corr
LE303	*Chironomid (10-day)*	DeChlor (100%)	10:1	192 (110 - 373)	110 (5.9 - 216)	80 (-24 - 185)	0.528
LE303	*Hexagenia (21-day)*	DeChlor (100%)	10:1	N.I.	N.I.	N.I.	
LE303	*Hyalella (28-day)*	DeChlor (100%)	67:1	948 (573 - 1327)	547 (158 - 935)	396 (-1.1 - 791)	0.385
Long Point	*Chironomid (10-day)*	DeChlor (100%)	10:1	619 (456 - 784)	328 (182 - 473)	232 (98 - 354)	0.853
Long Point	*Hexagenia (21-day)*	DeChlor (100%)	4:1	2994 (1850 - 4144)	1098 (150 - 2040)	608 (-127 - 1340)	0.691
Long Point	*Hyalella (28-day)*	DeChlor (100%)	67:1	2183 (1876 - 2491)	1562 (1124 - 2007)	1288 (798 - 1778)	0.547
Long Point	*Tubifex (28-day)*	DeChlor (100%)	10:1	N.I.	N.I.	N.I.	NA
Restoule	*Hyalella (28-day)*	DeChlor (100%)	67:1	N.I.	N.I.	N.I.	NA
Restoule	*Hyalella (28-day)*	DeChlor (100%)	200:1	N.I.	N.I.	N.I.	NA
Restoule	*Hyalella (28-day)*	DeChlor (100%)	500:1	N.I.	N.I.	N.I.	NA
Restoule	*Hyalella (28-day)*	DeChlor (100%)	1000:1	N.I.	N.I.	N.I.	NA
Restoule	*Hyalella (28-day)*	DeChlor (10%)	67:1	C.N.C.	C.N.C.	C.N.C.	NA
Restoule	*Hyalella (28-day)*	DeChlor (10%)	200:1	C.N.C.	C.N.C.	C.N.C.	NA
Restoule	*Hyalella (28-day)*	DeChlor (10%)	500:1	N.I.	N.I.	N.I.	NA
Restoule	*Hyalella (28-day)*	DeChlor (10%)	1000:1	C.N.C.	C.N.C.	C.N.C.	NA

N.I. - No Impact
C.N.C. - Could Not Calculate

Table 7. Critical Zn sediment concentrations (ug•g^{-1} dry wt) at 50, 20 and 10 percent reduction in growth **(dry wight)** with 95% confidence limits in exposures of the four test organisms to different sediments and overlay waters. Corrected R^2 indicates the relative predictive power of the growth model.

				Zn µg•g^{-1} dry wt			
Sediment	Organism	Overlay Water	Water:Sediment	EC50	EC20	EC10	R^2 Corr
LE303	*Chironomid (10-day)*	DeChlor (100%)	10:1	275 (216 - 334)	197 (107 - 287)	162 (52 - 273)	0.459
LE303	*Hexagenia (21-day)*	DeChlor (100%)	10:1	N.I.	N.I.	N.I.	
LE303	*Hyalella (28-day)*	DeChlor (100%)	67:1	817 (400 - 1235)	387 (-26 - 798)	250 (-122 - 622)	0.428
Long Point	*Chironomid (10-day)*	DeChlor (100%)	10:1	N.I.	N.I.	N.I.	
Long Point	*Hexagenia (21-day)*	DeChlor (100%)	4:1	3177 (1726 - 4628)	1235 (-52 - 2523)	713 (-341 - 1765)	0.572
Long Point	*Hyalella (28-day)*	DeChlor (100%)	67:1	2595 (2262 - 2929)	1876 (1268 - 2491)	1556 (804 - 2314)	0.477
Long Point	*Tubifex (28-day)*	DeChlor (100%)	10:1	N.I.	N.I.	N.I.	NA
Restoule	*Hyalella (28-day)*	DeChlor (100%)	67:1	N.I.	N.I.	N.I.	NA
Restoule	*Hyalella (28-day)*	DeChlor (100%)	200:1	N.I.	N.I.	N.I.	NA
Restoule	*Hyalella (28-day)*	DeChlor (100%)	500:1	N.I.	N.I.	N.I.	NA
Restoule	*Hyalella (28-day)*	DeChlor (100%)	1000:1	N.I.	N.I.	N.I.	NA
Restoule	*Hyalella (28-day)*	DeChlor (10%)	67:1	C.N.C.	C.N.C.	C.N.C.	NA
Restoule	*Hyalella (28-day)*	DeChlor (10%)	200:1	C.N.C.	C.N.C.	C.N.C.	NA
Restoule	*Hyalella (28-day)*	DeChlor (10%)	500:1	N.I.	N.I.	N.I.	NA
Restoule	*Hyalella (28-day)*	DeChlor (10%)	1000:1	C.N.C.	C.N.C.	C.N.C.	NA

N.I. - No Impact
C.N.C. - Could Not Calculate

Table 8. Critical Zn overlay water concentrations (ug•L^{-1}) at 50, 20 and 10 percent mortality with 95% confidence limits in exposures of the four test organisms to different sediments and overlay waters. Corrected R^2 indicates the relative predictive power of the mortality model.

				Zn ug•L^{-1}			
Sediment	Organism	Overlay Water	Water:Sediment	LC50 (95% C.I.)	LC20 (95% C.I.)	LC10 (95% C.I.)	R^2 Corr
LE303	*Chironomid (10-day)*	DeChlor (100%)	10:1	46.1 (34.3 - 57.9)	37.7 (24.6 - 50.7)	33.6 (20.4 - 46.9)	0.719
LE303	*Hexagenia (21-day)*	DeChlor (100%)	10:1	N.I.	N.I.	N.I.	NA
LE303	*Hyalella (28-day)*	DeChlor (100%)	67:1	94.1 (68.6 - 90.2)	61.8 (42.6 - 81.1)	50.2 (33.6 - 66.7)	0.784
Long Point	*Chironomid (10-day)*	DeChlor (100%)	10:1	66.0 (39.9 - 92.2)	38.9 (22.3 - 55.4)	30.5 (16.9 - 44.1)	0.738
Long Point	*Hexagenia (21-day)*	DeChlor (100%)	4:1	N.I.	N.I.	N.I.	NA
Long Point	*Hyalella (28-day)*	DeChlor (100%)	67:1	235 (134 - 335)	160 (75.8 - 245)	133 (56.4 - 209)	0.641
Long Point	*Tubifex (28-day)*	DeChlor (100%)	10:1	N.I.	N.I.	N.I.	
Restoule	*Hyalella (28-day)*	DeChlor (100%)	67:1	309 (158 - 459)	222 (90.2 - 354)	187 (66.0 - 308)	0.615
Restoule	*Hyalella (28-day)*	DeChlor (100%)	200:1	97.4 (-360 - 554)	44.8 (-180 - 271)	33.0 (-139 - 205)	0.493
Restoule	*Hyalella (28-day)*	DeChlor (100%)	500:1	77.8 (-24.2 - 179)	47.7 (-19.6 - 115)	37.9 (-18.2 - 94.1)	0.734
Restoule	*Hyalella (28-day)*	DeChlor (100%)	1000:1	63.3 (-8.89 - 135)	40.8 (-10.6 - 92.2)	32.9 (-10.1 - 75.8)	0.833
Restoule	*Hyalella (28-day)*	DeChlor (10%)	67:1	---------- C.N.C. (100% Mortality in all treatments) ---------			
Restoule	*Hyalella (28-day)*	DeChlor (10%)	200:1	---------------- C.N.C. (Poor Control Survival) ----------------			
Restoule	*Hyalella (28-day)*	DeChlor (10%)	500:1	38.9 (-113 - 191)	26.1 (-86.9 - 140)	21.7 (-75.8 - 119)	0.572
Restoule	*Hyalella (28-day)*	DeChlor (10%)	1000:1	---------------- C.N.C. (Poor Control Survival) ----------------			

N.I. - No Impact
C.N.C. - Could Not Calculate

Table 9. Critical Zn overlay water concentrations (ug•L^{-1}) at 50, 20 and 10 percent reduction in growth (**wet wight**) with 95% confidence limits in exposures of the four test organisms to different sediments and overlay waters. Corrected R^2 indicates the relative predictive power of the growth model.

				Zn ug•L^{-1}			
Sediment	Organism	Overlay Water	Water:Sediment	EC50	EC20	EC10	R^2 Corr
LE303	*Chironomid (10-day)*	DeChlor (100%)	10:1	21.9 (8.17 - 35.6)	13.1 (-1.18 - 27.5)	9.81 (-3.86 - 23.5)	0.478
LE303	*Hexagenia (21-day)*	DeChlor (100%)	10:1	N.I.	N.I.	N.I.	
LE303	*Hyalella (28-day)*	DeChlor (100%)	67:1	110 (44.5 - 175)	52.9 (-10.3 - 116)	34.6 (-22.3 - 91.5)	0.385
Long Point	*Chironomid (10-day)*	DeChlor (100%)	10:1	9.67 (1.11 - 18.2)	2.88 (-0.92 - 6.67)	1.40 (-0.81 - 3.61)	0.850
Long Point	*Hexagenia (21-day)*	DeChlor (100%)	4:1	477 (-622 - 1575)	36.5 (-135 - 208)	8.11 (-41.7 - 58.0)	0.681
Long Point	*Hyalella (28-day)*	DeChlor (100%)	67:1	229 (96.1 - 362)	70.6 (-23.3 - 165)	35.4 (-32.6 - 103)	0.442
Long Point	*Tubifex (28-day)*	DeChlor (100%)	10:1	N.I.	N.I.	N.I.	
Restoule	*Hyalella (28-day)*	DeChlor (100%)	67:1	N.I.	N.I.	N.I.	NA
Restoule	*Hyalella (28-day)*	DeChlor (100%)	200:1	N.I.	N.I.	N.I.	NA
Restoule	*Hyalella (28-day)*	DeChlor (100%)	500:1	N.I.	N.I.	N.I.	NA
Restoule	*Hyalella (28-day)*	DeChlor (100%)	1000:1	N.I.	N.I.	N.I.	NA
Restoule	*Hyalella (28-day)*	DeChlor (10%)	67:1	C.N.C.	C.N.C.	C.N.C.	NA
Restoule	*Hyalella (28-day)*	DeChlor (10%)	200:1	C.N.C.	C.N.C.	C.N.C.	NA
Restoule	*Hyalella (28-day)*	DeChlor (10%)	500:1	N.I.	N.I.	N.I.	NA
Restoule	*Hyalella (28-day)*	DeChlor (10%)	1000:1	C.N.C.	C.N.C.	C.N.C.	NA

N.I. - No Impact
C.N.C. - Could Not Calculate

Table 10. Critical Zn overlay water concentrations (ug•L^{-1}) at 50, 20 and 10 percent reduction in growth (**dry wight**) with 95% confidence limits in exposures of the four test organisms to different sediments and overlay waters. Corrected R^2 indicates the relative predictive power of the growth model.

				Zn ug•L^{-1}			
Sediment	Organism	Overlay Water	Water:Sediment	EC50	EC20	EC10	R^2 Corr
LE303	*Chironomid (10-day)*	DeChlor (100%)	10:1	39.0 (21.6 - 56.5)	25.2 (0.13 - 50.2)	19.5 (-9.87 - 48.8)	0.267
LE303	*Hexagenia (21-day)*	DeChlor (100%)	10:1	N.I.	N.I.	N.I.	
LE303	*Hyalella (28-day)*	DeChlor (100%)	67:1	79.8 (-18.4 - 178)	28.6 (-42.0 - 99.4)	15.6 (-35.2 - 66.7)	0.422
Long Point	*Chironomid (10-day)*	DeChlor (100%)	10:1	N.I.	N.I.	N.I.	NA
Long Point	*Hexagenia (21-day)*	DeChlor (100%)	4:1	481 (-1026 - 1987)	33.7 (-181 - 248)	7.13 (-52.2 - 66.7)	0.576
Long Point	*Hyalella (28-day)*	DeChlor (100%)	67:1	C.N.C.	C.N.C.	C.N.C.	NA
Long Point	*Tubifex (28-day)*	DeChlor (100%)	10:1	N.I.	N.I.	N.I.	
Restoule	*Hyalella (28-day)*	DeChlor (100%)	67:1	N.I.	N.I.	N.I.	NA
Restoule	*Hyalella (28-day)*	DeChlor (100%)	200:1	N.I.	N.I.	N.I.	NA
Restoule	*Hyalella (28-day)*	DeChlor (100%)	500:1	N.I.	N.I.	N.I.	NA
Restoule	*Hyalella (28-day)*	DeChlor (100%)	1000:1	N.I.	N.I.	N.I.	NA
Restoule	*Hyalella (28-day)*	DeChlor (10%)	67:1	C.N.C.	C.N.C.	C.N.C.	NA
Restoule	*Hyalella (28-day)*	DeChlor (10%)	200:1	C.N.C.	C.N.C.	C.N.C.	NA
Restoule	*Hyalella (28-day)*	DeChlor (10%)	500:1	N.I.	N.I.	N.I.	NA
Restoule	*Hyalella (28-day)*	DeChlor (10%)	1000:1	C.N.C.	C.N.C.	C.N.C.	NA

N.I. - No Impact
C.N.C. - Could Not Calculate

Table 11. Critical Zn body concentrations (ug•g^{-1} dry wt.) at 50, 20 and 10 percent mortality with 95% confidence limits in exposures of the four test organisms to different sediments and overlay waters. Corrected R^2 indicates the relative predictive power of the mortality model.

				Zn ug•g^{-1} dry wt			
Sediment	Organism	Overlay Water	Water:Sediment	LC50 (95% C.I.)	LC20 (95% C.I.)	LC10 (95% C.I.)	R^2 Corr
LE303	*Chironomid (10-day)*	DeChlor (100%)	10:1	C.N.C.	C.N.C.	C.N.C.	N.I.
LE303	*Hexagenia (21-day)*	DeChlor (100%)	10:1	N.I.	N.I.	N.I.	NA
LE303	*Hyalella (28-day)*	DeChlor (100%)	67:1	477 (C.N.C.)	469 (425 -513)	464 (392 - 537)	0.017
Long Point	*Chironomid (10-day)*	DeChlor (100%)	10:1	C.N.C.	C.N.C.	C.N.C.	NA
Long Point	*Hexagenia (21-day)*	DeChlor (100%)	4:1	C.N.C.	C.N.C.	C.N.C.	NA
Long Point	*Hyalella (28-day)*	DeChlor (100%)	67:1	752 (-11270 - 13139)	135 (-53.5 - 325)	87.6 (5.16 - 170)	0.047
Long Point	*Tubifex (28-day)*	DeChlor (100%)	10:1	N.I.	N.I.	N.I.	NA
Restoule	*Hyalella (28-day)*	DeChlor (100%)	67:1	235 (226 - 243)	228 (220 - 236)	224 (215 - 232)	0.340
Restoule	*Hyalella (28-day)*	DeChlor (100%)	200:1	C.N.C.	C.N.C.	C.N.C.	NA
Restoule	*Hyalella (28-day)*	DeChlor (100%)	500:1	C.N.C.	C.N.C.	C.N.C.	NA
Restoule	*Hyalella (28-day)*	DeChlor (100%)	1000:1	C.N.C.	C.N.C.	C.N.C.	NA
Restoule	*Hyalella (28-day)*	DeChlor (10%)	67:1	------------------- 100% Mortality, even in the Controls -------------------			
Restoule	*Hyalella (28-day)*	DeChlor (10%)	200:1		C.N.C.		NA
Restoule	*Hyalella (28-day)*	DeChlor (10%)	500:1	C.N.C.	C.N.C.	C.N.C.	NA
Restoule	*Hyalella (28-day)*	DeChlor (10%)	1000:1		C.N.C.		NA

N.I. - No Impact
C.N.C. - Could Not Calculate

Table 12. Critical Zn body concentrations (ug•g^{-1} dry wt.) with 95% confidence limits, associated with 50, 20 and 10 percent reduction in growth (**wet wight**) in exposures of the four test organisms to different sediments and overlay waters. Corrected R^2 indicates the relative predictive power of the growth model.

				Zn ug•g^{-1} dry wt			
Sediment	Organism	Overlay Water	Water:Sediment	EC50	EC20	EC10	R^2 Corr
LE303	*Chironomid (10-day)*	DeChlor (100%)	10:1	308 (-380 - 994)	111 (-361 - 582)	61.0 (-273 - 395)	0.432
LE303	*Hexagenia (21-day)*	DeChlor (100%)	10:1	NA	NA	NA	NA
LE303	*Hyalella (28-day)*	DeChlor (100%)	67:1	N.I.	N.I.	N.I.	NA
Long Point	*Chironomid (10-day)*	DeChlor (100%)	10:1	238 (180 - 297)	135 (69.3 - 201)	97.4 (34.6 - 160)	0.844
Long Point	*Hexagenia (21-day)*	DeChlor (100%)	4:1	175 (87.6 - 263)	100 (-2.61 - 201)	70.6 (-47.1 - 189)	0.219
Long Point	*Hyalella (28-day)*	DeChlor (100%)	67:1	131 (93.5 - 169)	87.6 (24.2 - 150)	68.6 (-3.27 - 141)	0.293
Long Point	*Tubifex (28-day)*	DeChlor (100%)	10:1	C.N.C.	C.N.C.	C.N.C.	NA
Restoule	*Hyalella (28-day)*	DeChlor (100%)	67:1	C.N.C.	C.N.C.	C.N.C.	NA
Restoule	*Hyalella (28-day)*	DeChlor (100%)	200:1	C.N.C.	C.N.C.	C.N.C.	NA
Restoule	*Hyalella (28-day)*	DeChlor (100%)	500:1	C.N.C.	C.N.C.	C.N.C.	NA
Restoule	*Hyalella (28-day)*	DeChlor (100%)	1000:1	C.N.C.	C.N.C.	C.N.C.	NA
Restoule	*Hyalella (28-day)*	DeChlor (10%)	67:1	C.N.C.	C.N.C.	C.N.C.	NA
Restoule	*Hyalella (28-day)*	DeChlor (10%)	200:1	C.N.C.	C.N.C.	C.N.C.	NA
Restoule	*Hyalella (28-day)*	DeChlor (10%)	500:1	C.N.C.	C.N.C.	C.N.C.	NA
Restoule	*Hyalella (28-day)*	DeChlor (10%)	1000:1	C.N.C.	C.N.C.	C.N.C.	NA

N.I. - No Impact
C.N.C. - Could Not Calculate

Table 13. Critical Zn body concentrations (ug•g^{-1} dry wt.) at 50, 20 and 10 percent reduction in growth **(dry wight)** with 95% confidence limits in exposures of the four test organisms to different sediments and overlay waters. Corrected R^2 indicates the relative predictive power of the growth model.

				Zn ug•g^{-1} dry wt			
Sediment	Organism	Overlay Water	Water:Sediment	EC50	EC20	EC10	R^2 Corr
LE303	*Chironomid (10-day)*	DeChlor (100%)	10:1	634 (395 - 876)	371 (48.8 - 693)	271 (-89.6 - 633)	0.402
LE303	*Hexagenia (21-day)*	DeChlor (100%)	10:1	NA	NA	NA	NA
LE303	*Hyalella (28-day)*	DeChlor (100%)	67:1	N.I.	N.I.	N.I.	NA
Long Point	*Chironomid (10-day)*	DeChlor (100%)	10:1	N.I.	N.I.	N.I.	NA
Long Point	*Hexagenia (21-day)*	DeChlor (100%)	4:1	165 (101 - 228)	107 (13.1 - 201)	83.0 (-35.3 - 201)	0.227
Long Point	*Hyalella (28-day)*	DeChlor (100%)	67:1	N.I.	N.I.	N.I.	NA
Long Point	*Tubifex (28-day)*	DeChlor (100%)	10:1	N.I.	N.I.	N.I.	NA
Restoule	*Hyalella (28-day)*	DeChlor (100%)	67:1	C.N.C.	C.N.C.	C.N.C.	NA
Restoule	*Hyalella (28-day)*	DeChlor (100%)	200:1	C.N.C.	C.N.C.	C.N.C.	NA
Restoule	*Hyalella (28-day)*	DeChlor (100%)	500:1	C.N.C.	C.N.C.	C.N.C.	NA
Restoule	*Hyalella (28-day)*	DeChlor (100%)	1000:1	C.N.C.	C.N.C.	C.N.C.	NA
Restoule	*Hyalella (28-day)*	DeChlor (10%)	67:1	C.N.C.	C.N.C.	C.N.C.	NA
Restoule	*Hyalella (28-day)*	DeChlor (10%)	200:1	C.N.C.	C.N.C.	C.N.C.	NA
Restoule	*Hyalella (28-day)*	DeChlor (10%)	500:1	C.N.C.	C.N.C.	C.N.C.	NA
Restoule	*Hyalella (28-day)*	DeChlor (10%)	1000:1	C.N.C.	C.N.C.	C.N.C.	NA

N.I. - No Impact
C.N.C. - Could Not Calculate

3.6 Total Organic Carbon Impact on *H. azteca* LC50

LC50s, calculated from 28-day exposures of juvenile *H. azteca* to LE303, Long Point and Restoule spiked sediments with hard (100% DeChlor) overlay water at a 67:1 water to sediment ratio (Table 5), were regressed against total organic carbon (Table 1) measured in each sediment type (Fig. 16). Toxicity was reduced (LC50 increased) with increasing total organic content such that the LC50 value increased by 18.9 ug Zn•mg^{-1} TOC per one gram dry sediment. There was no relationship between LC50s and other sediment constituents measured (clay, silt or sand).

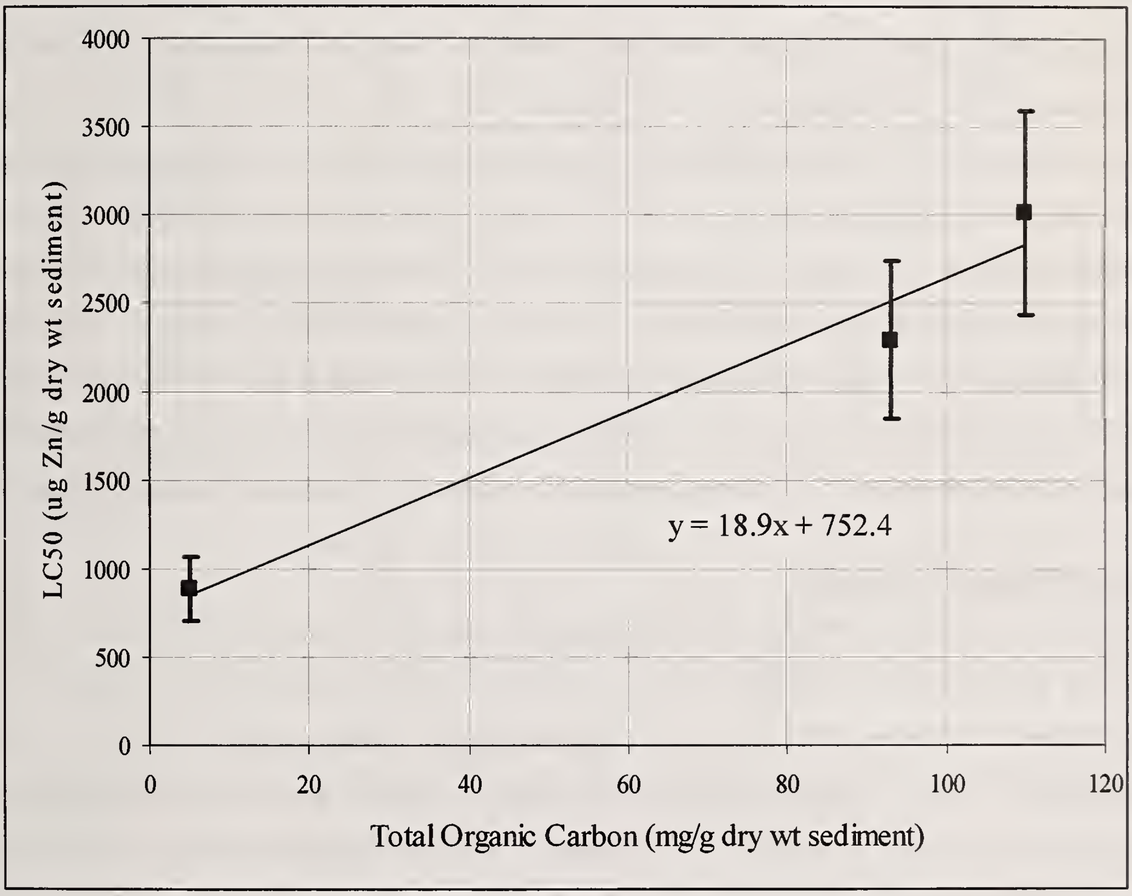


Figure 16. *H. azteca* LC50 (ug Zn•g^{-1} dry wt sediment) versus total organic carbon (mg•g^{-1}) in each sediment. Error bars represent 95% confidence limits.

3.7 Test Ratio (Overlay Water:Sediment Volume) Impact on *H. azteca* LC50

During the toxicity testing of Restoule Zn-spiked sediments with soft overlay water (10% DeChlor), there was 100% mortality at all Zn concentrations (including the controls) when the tests were conducted at a 67:1 ratio of overlay water to sediment volume. Although *H. azteca* inhabit Lake Restoule (Shuhaimi-Othman *et al.*, 2006) the buffering capacity of the soft overlay water may not have been enough for survival in our cone tests, even at the large water-sediment ratios that the cone methods

provide (Borgmann and Norwood, 1999). This was reflected by the mean pH levels, which were as much as a unit lower than at the other sites when tested at the 67:1 water to sediment ratio (Appendix 1). Therefore, single replicates of each Zn concentrations in a series of larger water:sediment ratios (200:1, 500:1 & 1000:1) were tested with the soft (10% DeChlor) overlay waters. Mean pH improved (increased), total ammonia levels improved (decreased) and conductivity decreased (indicating lower concentration of ions in solution) with increased ratio (Appendix 1). *Hyalella azteca* survival improved at all ratios greater than the 67:1 (Fig. 11). Unfortunately, the 200:1 and the 1000:1 treatments were still not acceptable since the control survivals were too low, but the 500:1 ratio test was acceptable. Single replicates of larger water:sediment ratios were also tested with hard (100% DeChlor) for comparison. Toxicity decreased (LC50 increased) with increasing hard water:sediment ratio (Fig. 17). However, even though LC50 increased by a factor of 2, it was not statistically significant since there was a high level of variation in the LC50s for the 200:1, 500:1 and 1000:1 ratios, given that there were only single replicates of the Zn concentrations tested in each of the ratio treatments. The 67:1 test had 4 replicates per Zn concentrations and hence the LC50 had a much smaller 95% confidence interval (Fig. 17, Table 5). The soft overlay water at the 500:1 ratio of water to sediment generated a LC50 value that was 4 times lower (more toxic) than that in hard water (Fig. 17). Again, the variation was large for this value due to only one replicate per Zn concentration and therefore, the soft and hard LC50s at the 500:1 ratio were not significantly different.

3.8 Other Trace Elements

Cadmium, Co, Ga Mn Mo, Ni, Tl, W and Zn concentrations increased in the hard and/or the soft overlay water of the highest Zn-spiked sediment compared to that of the overlay waters of the control sediment (Table 14, Enrichment Factor >2.0). Concentrations of other elements such as Al, As, Be, Ce, Cr, Fe, Ga, La, U, V, and Y were more than 50% lower in the hard and/or soft overlay water with the highest Zn-spiked sediments compared to the control (Table 14, Enrichment Factor <0.5). There was a more than 2-fold increase in Al, As, B, Co, Cs, Cu, Ga Li, Mo, Rb, Sb, Se, Sn, Sr, U, V, and W concentrations in the hard overlay water in a comparison to the soft overlay waters of the control sediment and/or the highest Zn-spiked sediments (Table 14, Hardness Factor >2.0). Alternately, Be, Cd, Ce, La, Ni, Tl, Y and Zn concentrations were more than 50% lower in the hard overlay waters in comparison to the soft overlay waters of the control and/or the highest Zn-spiked sediments (Table 14, Hardness Factor <0.5).

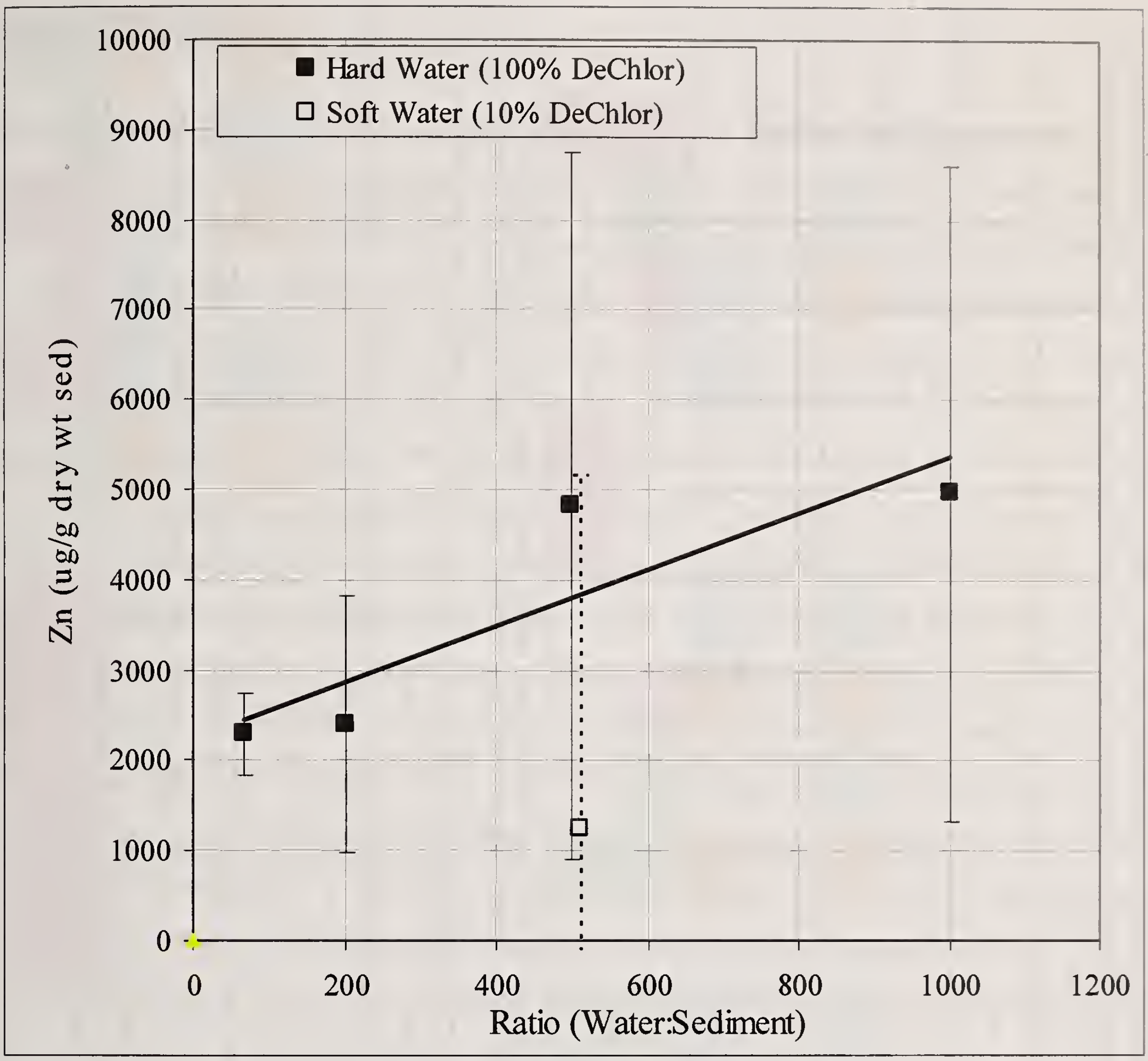


Figure 17. *H. azteca* LC50 (ug Zn•g^{-1} dry wt sediment) versus ratio of overlay water volume to sediment volume. Error bars represent 95% confidence limits.

Table 14: Trace element concentrations ($\mu g \cdot L^{-1}$) in soft (10%) and hard (100%) overlay water (de-chlorinated city of Burlington tap water from Lake Ontario) exposed to control and Zn-spiked Lake Restoule sediments. Enrichment factor was calculated as the element concentration in the Zn-spiked sediment overlay water divided by the concentrations in the control sediment overlay water. The hardness factor was calculated by dividing the concentration of an element in the 100% DeChlor overlay water by the concentration in the 10% DeChlor overlay water. ICP-MS Method detection limits are listed for each element.

Overlay Water	Sediment	Zn nmol•g^{-1} dry wt	Ag	Al	As	B	Ba	Be	Bi	Cd	Ce	Co	Cr	Cs	Cu	Fe	Ga	La	Li
10% DeChlor	Control	3870	<D.L.	22.5	0.599	6.03	41.5	0.0112	<D.L.	0.157	0.248	0.02	0.333	0.0120	1.19	4.44	0.0047	0.191	0.497
	Zn-Spiked	339035	<D.L.	2.8	0.209	4.75	32.1	0.0024	<D.L.	0.940	0.080	2.02	0.096	0.0118	0.68	2.90	0.0249	0.054	0.528
	Enrichment Factor	87	NA	0.1	0.3	0.8	0.8	0.2	NA	6.0	0.3	111	0.3	1.0	0.6	0.7	5.3	0.3	1.1
100% DeChlor	Control	3870	<D.L.	55.8	0.663	26.5	40.0	0.0026	0.0016	0.026	0.063	0.04	0.481	0.0128	2.29	2.70	0.129	0.0631	1.73
	Zn-Spiked	339035	<D.L.	7.5	0.445	25.73	57.0	0.0010	<D.L.	0.484	0.095	3.53	0.157	0.0232	2.35	1.17	0.0350	0.065	2.411
	Enrichment Factor	87	NA	0.1	0.7	1.0	1.4	0.4	NA	19	1.5	84	0.3	1.8	1.0	0.4	0.3	1.0	1.4
	Hardness Factor Controls		NA	2.5	1.1	4.4	1.0	0.2	NA	0.2	0.3	2.3	1.4	1.1	1.9	0.6	27	0.3	3.5
	Hardness Factor Spiked		NA	2.6	2.1	5.4	1.8	0.4	NA	0.5	1.2	1.7	1.6	2.0	3.4	0.4	1.4	1.2	4.6
Detection Limit ($\mu g \cdot L^{-1}$)			0.0160	0.361	0.00661	0.0289	0.0460	0.00073	0.00098	0.00055	0.00212	0.00060	0.00211	0.00199	0.0338	0.0998	0.00059	0.00221	0.0115

Overlay Water	Sediment	Zn nmol•g^{-1} dry wt	Mn	Mo	Nb	Ni	Pb	Pt	Rb	Sb	Se	Sn	Sr	Ti	Tl	U	V	W	Y	Zn
10% DeChlor	Control	3870	41	0.025	<D.L.	1.53	0.0686	<D.L.	1.24	0.079	0.093	0.0053	36.4	0.0351	0.0380	0.005	0.285	0.0002	0.0938	21
	Zn-Spiked	339035	595	0.112	<D.L.	4.62	0.0444	<D.L.	1.08	0.043	0.173	0.0070	32.3	0.0498	0.0780	0.002	0.252	0.0054	0.0180	8348
	Enrichment Factor	87	15	4.6	NA	3.0	0.6	NA	0.9	0.5	1.9	1.3	0.9	1.4	2.1	0.4	0.9	27	0.2	403
100% DeChlor	Control	3870	1.1	1.48	<D.L.	0.71	0.0825	<D.L.	2.52	0.209	0.217	0.0145	191	0.0299	0.0159	0.189	0.640	0.0234	0.0248	11
	Zn-Spiked	339035	972	1.43	<D.L.	1.97	0.0862	<D.L.	2.60	0.145	0.370	0.0153	197	0.0292	0.0776	0.076	0.261	0.0441	0.0354	706
	Enrichment Factor	87	869	1.0	NA	2.8	1.0	NA	1.0	0.7	1.7	1.1	1.0	1.0	4.9	0.4	0.4	1.9	1.4	64
Hardness Factor Controls			0.03	60	NA	0.46	1.2	NA	2.0	3	2	2.7	5	0.85	0.42	38	2.2	117	0.26	0.5
Hardness Factor Zn-Spiked			1.6	13	NA	0.43	1.9	NA	2.4	3	2	2.2	6	0.6	1.0	42	1.0	8	1.97	0.1
Detection Limit ($\mu g \cdot L^{-1}$)			0.187	0.00823	0.00008	0.0341	0.0137	0.00018	0.00416	0.00046	0.0540	0.00175	0.0437	0.00486	0.00385	0.00175	0.00251	0.00010	0.00050	0.124

Enrichment Factor > 2 fold increase in element water concentration with increased sediment Zn
Enrichment Factor < 0.5 fold change in element concentration with increased sediment Zn
Hardness Factor > 2 fold increase in element concentration with increased hardness
Hardness Factor < 0.5 fold change in element concentration with increased hardness

4 DISCUSSION

This study has generated spiked sediment toxicity data to support the development of a full Canadian SQG for Zn. In total, 16 Zn sediment toxicity tests were conducted with 4 test organisms and 3 sediment types. Toxicity varied as a function of sediment organic carbon, grain size, water:sediment ratio, and/or test species. Due to the spiking method, there was a reduction in Zn sediment concentrations from the pre-spiking levels (Table 2) to the post spiking concentrations in the control treatments (Table 3). The spiking method used, which involved the mixing of the test sediment with the spike solution (50:50 v:v), and subsequent decanting of the excess water after the sediment was allowed to settle back down, effectively "washed" the sediment such that any ion or dissolved material would be decanted with the water. Decreased Zn concentrations were observed in the control treatments, but other components of the sediment such as organic carbon, fine particulates, other ions, AVS, etc. may have also changed. Since a re-analysis of these parameters was not performed post-spike, the exact composition of the sediment post-spiking was not known. However, it is assumed that the relative difference between the three sediment types likely remained the same and the sediment with the highest organic content pre-spiking still had the highest organic content post-spiking, etc. The same effect would occur if the sediments were sieved to remove any indigenous organisms as was the case for the Long Point sediment (this sediment was in essence washed twice, once while sieving and the second time during the spiking process).

Some data could not be used in the derivation of the critical lethal or effect concentrations and not all tests were conducted on all three sediment types. Sieving was not done with the LE303 sediment and it turned out that the sediment contained a substantial population of indigenous oligochaete worms, therefore, the *T. tubifex* reproduction test could not be performed on this sediment. The *Hexagenia* spp. test is primarily a growth test, but unfortunately wet weights were not measured in the LE303 test. *Chironomus riparius* testing was performed with LE303 and Long Point spiked sediments but not with Restoule spiked sediments. The *H. azteca* test was conducted with all three sediment types and could be used to compare the three sediments. However, poor control survival or 100% mortality occurred in some treatments with Lake Restoule sediment when a soft overlay water was used, and therefore data was limited in these cases.

Therefore the four test organism sensitivities can be compared in the following manner; 1) survival (lethal Zn concentrations) can be compared between *C. riparius*, and *H. azteca* with the LE303 and Long Point Zn-spiked sediment tests, 2) growth (Zn effect concentrations) can be compared between *C. riparius* and *H. azteca* with the LE303 sediment and between *C. riparius*, *H. azteca* and *Hexagenia* spp. with Long Point Zn-spiked sediment tests, 3) *T. tubifex* reproduction (effect concentration) can be evaluated only with Long Point sediment, and 4) *H. azteca* survival (lethal concentration) and growth

(effect concentration) can be used to evaluate all sediments, the effect of hard versus soft water with Restoule sediments, and determine the effect of different water:sediment ratios with Restoule sediments.

Table 15. Rank of organism sensitivity based on critical sediment concentrations ($ug \cdot g^{-1}$ dry wt.) with 95% confidence interval in brackets. Values with different superscript letter are statistically different ($p<0.05$).

Organism	Rank	LC10 (95% C.I.)	EC10 (95% C.I.)
C.riparius	1	269 (167 - 357) ^{a}	80 (-24 - 185) ^{a}
H. azteca	2	526 (385 - 667) ^{b}	396 (-1.1 - 791) $^{a\,b}$
Hexagenia spp.	3	NA	608 (-127 - 1340) $^{a\,b}$
T. tubifex	4	NA	>2730 ^{c}
ISQG (freshwater)		123	(CCME, 1999)
PEL (freshwater)		315	(CCME, 1999)

NA - Not Applicable

Chironomus. riparius was the most sensitive organism tested overall, followed by *H. azteca* and *Hexagenia* spp., with the least sensitive being *T. tubifex* (Table 15). It is also apparent from Table 15 that even though the effect concentrations based on growth were lower than the lethal concentrations, they were more variable than the lethal concentrations. The *C. riparius* EC10 and EC20 of 80 and 110 ug $Zn \cdot g^{-1}$ dry wt, respectively, for LE303 (Table 6) were lower than the ISQG (Table 15) and even its EC50 of 192 ug $Zn \cdot g^{-1}$ dry wt was less than the PEL. Also, the *C. riparius* LC10 and LC20 (Table 5) fell between the ISQG and the PEL and its LC50 was less than two times the PEL. The Long Point Zn-spiked sediments were less toxic to *C. riparius*, such that its EC10 of 232 ug $Zn \cdot g^{-1}$ dry wt fell between the ISQG and the PEL and its EC20 and EC50 were less than two times the PEL (Table 6). It is possible that all of the point estimates for *C. riparius* may have been even lower if it had been tested using soft overlay water with the spiked sediments, especially from Lake Restoule, as was observed with *H. Azteca*. For *H. azteca*, the LC10 and LC20 of 526 and 628 ug $Zn \cdot g^{-1}$ dry wt respectively and the EC10 and EC20 of 396 and 547 ug $Zn \cdot g^{-1}$ dry wt respectively with Zn-spiked LE303 sediments were less than two times the PEL (Tables 5 & 6). For *Hexagenia* spp., the EC10 of 608 ug $Zn \cdot g^{-1}$ dry wt (Table 6) was also less than two times the PEL.

Table 16. Rank of sediment toxicity based on *H. azteca* LC10 and EC10 (ug•g-1 dry wt.) with 95% confidence interval in brackets. Values with different superscript letter are statistically different ($p<0.05$).

Sediment	Rank	LC10 (95% C.I.)	EC10 (95% C.I.)
LE303 (Hard, 67:1)	1	526 (385 - 667) ^{a}	396 (-1.1 - 791) ^{a}
Restoule (Soft, 500:1)	2	758 (394 - 1120) ^{a}	N.I.
Restoule (Hard, 67:1)	3	1710 (1180 - 2240) ^{bc}	N.I.
Long Point (Hard, 67:1)	4	2110 (1510 - 2710) ^{c}	608 (-127 - 1340) ^{ab}

N.I. - No Impact

The LE303 spiked sediment appears to be the most toxic (Table 16), however the Restoule sediment tested at the 67:1 ratio of water to sediment, using soft (10% DeChlor) overlay water was very toxic, even in the controls. Unfortunately the fact that there was no survival in the controls invalidates the test. The 10% DeChlor overlay water had major ion concentrations (Ca 3.5, Mg 0.88, Na 1.4 and K 0.17 mg•L^{-1}) similar to those found in Lake Restoule (Ca 3.24, Mg 0.93, Na 1.85 and K 0.61 mg•L^{-1}) as determined by Borgmann *et al.* (2001b) from water samples collected 1 m of the bottom at the deepest location in the lake. There were *H. azteca* living in this lake (Shuhaimi-Othman *et al.*, 2006) and increased water:sediment ratios of 40:1, 100:1, 200:1 were used by Borgmann and Norwood (1999) to stabilize pH levels with Restoule sediments. This approach was repeated in this study with single replicates of all spike concentrations at different water:sediment ratios of 200:1, 500:1 and 1000:1, with both the hard and soft water.

Using this approach, the 500:1 ratio test with soft overlay water generated a LC10 similar to that for the LE303 test with the 67:1 ratio and hard overlay water (Table 5). The main point of this comparison is to point out that the testing of sediments from soft water lakes of the Canadian Shield can be difficult, but it is important that these sediments be tested with site water or laboratory water with ion concentrations similar to the site, in order to obtain a realistic assessment of the bioavailability and impact of Zn at the site. This is critical since the Canadian shield occupies approximately 50% of Canada and is a rich source of a number of metallic minerals including zinc.

Zinc bioaccumulation patterns by all of the species tested were not very useful in the prediction of effects (mortality or growth). A useful bioaccumulation pattern would first, show a continuous increase (significantly above background levels) in bioaccumulated Zn with increased exposure, to a possible maximum or saturation, such as in the bioaccumulation of cobalt by *H. azteca* (Norwood *et al.*, 2006). In that case there was a large range in cobalt body concentrations (0.59 to 39.7 ug•g^{-1} dry wt) with a 67-fold increase in bioaccumulation. Secondly, the bioaccumulation needs to be linked to an impact, such as increased mortality or decreased growth, as in the prediction of mortality with cobalt bioaccumulation

(Norwood *et al.*, 2007). There was only a two fold increase in Zn bioaccumulation by *H. azteca* exposed to LE303 spiked sediments (Fig. 6, lower panel), after which point there were no survival. These increased tissue concentrations were only observed at the highest exposure concentration. Similar patterns of accumulation were observed with the Long Point and the Restoule exposures (Fig. 6, upper panel and Fig. 7). It generally appears that *H. azteca* can regulate Zn and thus keep its Zn body concentrations at background levels across most of the exposure range. Critical tissue concentration could be calculated for *H. azteca* (Tables 11 and 12) but they were inconsistent and the variability was high (model prediction of $R^2<0.05$ for mortality and $R^2=0.29$ for growth).

Lethal body concentrations could not even be calculated for *C. raparius*, *Hexagenia* spp. or *T. Tubifex* (Table 11). However, *C. riparius* exhibited a 6.7 and 3.8 fold increases in tissue concentration above background with exposures to LE303 and Long Point sediments respectively (Fig. 6). There was no change in survival at these body concentrations and hence critical tissue concentrations could not be calculated, however once the mean body concentrations exceeded 607 and 335 ug•g^{-1} dry wt with LE303 and Long Point exposures respectively, there was no survival of *C. riparius*. *Hexagenia* spp. bioaccumulation with exposure to LE303 had a 11-fold increase in Zn bioaccumulation above background and thus this pattern should be adequate for calculating critical tissue concentrations (Fig. 6, upper panel). Unfortunately, the wet weight growth data was not collected so the critical tissue concentrations could not be calculated. The *Hexagenia* spp. test is primarily a growth test and there was no mortality with exposure to LE3203 or Long Point sediments. Zinc bioaccumulation in *T. tubifex* was interesting (Fig. 6, lower panel). There was little change in bioaccumulation with increased exposure until sediment Zn concentrations exceeded 1600 ug•g^{-1} dry wt. (Fig. 6, lower panel). At this point the body concentrations increased 19-fold, to a average maximum of 3100 ug•g^{-1} dry wt. above background, and there was a simultaneous drop in total offspring (Fig. 15). Therefore, when *T. tubifex* body concentration exceeded 219 ug•g^{-1} dry wt. with exposure to Long Point spiked sediments, there was a reduction in total offspring.

There were many other metals found at higher concentrations in the Restoule sediments (Table 2) compared to the Lake Erie sediments, even though Lake Restoule was considered a reference site by Borgmann *et al.*, (2001a, 2001b, 1998). The 100% mortality that occurred at all Restoule Zn concentrations for the test with soft water at the 67:1 ratio may have occurred due to low ion intolerance by *H. azteca* in combination with very low buffering and/or complexing capacity of the overlay water and hence toxic elements may have been readily available to the test organisms. The pH and conductivity was lower in the soft water treatments compared to the hard water treatments (Appendix 1 & 4) and total ammonia was higher in the soft water treatments compared to the hard water treatments (Appendix 2). The pH was at an acceptable neutral level but the low conductivity was indicative of low levels of other

ions. The total ammonia (0.18 mmol•L^{-1}) was lower than the 4-wk LC50 of 0.95 mmol•L^{-1} for total ammonia in 100% DeChlor, but higher than the LC50 of 0.076 mmol•L^{-1} in 10% DeChlor (Borgmann, 1994). Therefore, it is possible that total ammonia may have been partly responsible for the observed toxicity, however it is more likely that the ammonia levels increased due to the decomposition of excess food not consumed due to high mortality of the amphipods in this treatment. The total ammonia concentrations were 0 mmol•L^{-1} at the onset of the test. The Zn concentrations in the soft overlay water (Fig. 4, lower panel) were approximately 10-times higher than in the hard overlay water (Fig. 4, upper panel) in the 67:1 water:sediment ratio treatment and was as high as 30 ug•L^{-1} (Table 4). This concentrations was 5.5 times lower than the LC25 of 165 ug•L^{-1} reported by Borgmann *et al.* (2004), however, their were based on hard water exposure. The Zn may have been much more bioavailable in the soft water and thus toxicity increased.

The addition of Zn to a natural sediment affected other components of the sediment. The Zn may have out-competed certain elements (i.e., Cd, Co, Ga, Mn, Mo, Ni, Tl, W) for binding sites on the sediment and thereby these elements were released to the overlay water (enrichment of the water) whereas other elements (i.e., Al, As, Be, Ce, Cr, Fe, Ga, La, U, V, Y) remained more firmly bound to the sediments in the presence of the spiked Zn. The hardness of the water also displaced some elements (i.e, Al, As, B, Co, Cs, Cu, Ga Li, Mo, Rb, Sb, Se, Sn, Sr, U, V, W) from the sediment resulting in increased concentrations in the hard overlay water. Alternatively, increased hardness allowed other elements (i.e, Be, Cd, Ce, La, Ni, Tl, Y, Zn) to move into the sediments (both control and Zn-spiked).

In summary, the spiking of Zn into a sediment does not only lead to an increase in Zn in the sediment and the overlay water, but can also lead to shifts in the mobility (increased or decreased) of many other elements, some of which could be beneficial or detrimental. The spiking of a natural sediments with elevated levels of many elements, such as those from Lake Restoule, could lead to unexpected impacts or benefits. Similarly, the adjustment of hardness may cause a shift in the mobility of many elements, again leading to potentially unexpected impacts or benefits to an organism.

One of the reasons this work was undertaken was to produce SSTT results with a wider range of sediment types since in the past, published SSTT results for Zn reported the onset of toxicity at concentrations higher than those based on the co-occurrence approach, such as the current ISQG. The current ISQG and PELs are 123 and 315 ug•g^{-1} dry wt. respectively (CCME, 1999). According to CCME (1999, Protocol – Appendix B) a SQG should be derived from the lowest-observed-effect level (LOEL) of a SSTT. However, Environment Canada (2005) indicates that NOEC/LOEC calculation do not represent a good toxicological endpoint for the following reasons:

1. The endpoints are defined statistically rather than biologically; higher variability within the test leads to higher values of NOEC/LOEC.

2. The NOEC does not necessarily represent a safe level in the environment although it conveys that impression.
3. The endpoints can only be concentrations that were actually tested and are therefore open to manipulation by chance or design.
4. The calculations produce a pair of concentrations, rather than one endpoint.
5. No confidence limits can be calculated.

Instead, Environment Canada (2005) suggests that point estimates such as EC10, EC20, EC50 or LC10, LC20 and LC50 should be calculated. They also list a number of advantages of point estimates, with the major advantages as follows:

1. A single concentration is designated as the endpoint.
2. The endpoint can be any concentration within the range tested, but does not have to be a specific concentrations used in the test.
3. Confidence limits can be determined based on the entire data set across the "dose" range.

Therefore, the LC10 or EC10 is proposed as the appropriate endpoints for inclusion in a species sensitivity distribution from which a SQG can be derived. There were a number of other critical concentrations determined in this body of work, that were at or near the ISQG and the PEL.

The most toxic sediments in this study were from Lake Restoule when they were tested with soft overly water. Only tests employing a water to sediment ratio of 500:1 could be used in determinations of critical concentrations, however. For all other Restoule sediment treatments with soft water, critical concentrations could not be calculated due to complete mortality (67:1) or poor control survival (200:1, 1000:1). It was apparent that other factors contributed to the observed Toxicity at the 67:1 ratio with soft overlay water. Factors such as displacement of other ions, metals, lower pH, higher ammonia need to be taken into account when soft water sediments are being assessed.

5 SUMMARY AND CONCLUSIONS

1. Three sediments were tested that had a range of organic carbon and a range of grain size.
   a. The most toxic sediment was from Lake Restoule when tested with soft overlay water. However there were compounding factors such as other contaminants, lower pH and increased ammonia.
   b. The most toxic Zn-spiked sediment when tests were conducted with hard overlay water was LE303. It had the lowest organic carbon content and an intermediate amount of the smallest grain size (clay).

   c. The second most toxic Zn-spiked sediment, when tests were conducted with hard overlay water, was from Lake Restoule. Lake Restoule had a high organic content, the highest content of the smallest grain size (clay) and high load of metals.
   d. The least toxic Zn-spiked sediment, when tests were conducted with hard overlay water, was from Long Point. The Long Point sediment had the highest organic carbon content and was dominated by the intermediate grain sizes of silt and sand.
2. A number of critical concentrations were at or below the current ISQG
   a. The insect midge, *C. riparius*, was the most sensitive organism, for both sub-lethal and lethal endpoints. Growth was its most sensitive end point, but more variable, and the EC10 value was less than the ISQG. Its EC20, EC50, LC10 and LC20 values fell between the ISQG and the PEL.
   b. The epi-benthic crustacean, *H. azteca*, was the second most sensitive organism and growth was also its most sensitive endpoint, but again more variable. Its EC10, EC20, LC10 and LC20 were all less than 2 X the PEL.
   c. The insect, Hexagenia spp., was the third most sensitive, but only for growth. Its EC10 was less the 2 times the PEL. There was no impact on its survival.
   d. The benthic Oligochaete worm, *T. Tubifex*, was the least sensitive with no impact of the Zn-spiked Long Point sediment on survival or growth. There was a significant impairment of reproduction but only at the highest spike concentration.
3. Different water:sediment ratios had a number of impacts on the toxicity tests.
   a. In the standard tests with LE303 and Long Point Zn-spiked sediments, the overlay water Zn concentrations were very similar across the 4:1, 10:1 and 67:1 ratios, however, water quality parameters were somewhat better in the high 67:1 ratio (i.e. lower ammonia, improved oxygen, more stable pH).
   b. Increasing the water:sediment ratio from 67:1 to 200:1, 500:1 and 1000:1 enabled *H. azteca* to survive in very soft overlay waters with Lake Restoule Zn-spiked sediment. There was no survival in controls at the 67:1 ratio using the soft water.
4. Increased water hardness was protective of Zn toxicity to *H. azteca*. With LC10 values 2.3-3.4 times higher. Water concentrations were as much as 10 times higher in soft water compared to hard water with exposures to the same Zn-spiked sediments.
5. Bioaccumulation could not be modeled to predict effects. This was because the change in bioaccumulation for all test organisms was either too small compared to background or too variable, or the organism regulated the bioaccumulation level, or there was very little effects..

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Appendix 1

pH: mean, minimum, standard deviation and sample size (N) of measured pH values in overlay waters from the different; sediment treatments, organism tests, water to sediment ratios and water hardness (Restoule only).

		Chironomid 10:1				Hexagenia 10:1				Tubifex (indigenous) 10:1				Hyalella 67:1			
Site	NOM	Mean	Min	Std	N	Mean	Min	Std	N	Mean	Min	Std	N	Mean	Min	Std	N
L. Erie	0	7.8	7.2	0.69	10	8.4	8.4	0.00	2	8.5	8.4	0.06	3	8.5	8.4	0.16	2
303 with	180	7.9	7.3	0.65	7	8.5	8.5	0.00	2	8.4	8.4	0.00	2	8.4	8.4	0.05	2
100%	320	8.0	7.4	0.66	6	8.4	8.2	0.21	2	8.4	8.4	0.00	2	8.4	8.4	0.01	2
DeChlor	560	7.9	7.4	0.58	7	8.5	8.4	0.07	2	8.4	8.3	0.07	2	8.3	8.3	0.02	2
Overlay	1000	7.9	7.4	0.57	7	8.4	8.4	0.00	2	8.4	8.4	0.00	2	8.3	8.3	0.06	2
Water	1800	7.8	7.3	0.55	7	8.4	8.4	0.00	2	8.1	8.1	0.00	2	8.4	8.3	0.04	2
	3200	7.8	7.3	0.49	7	8.4	8.3	0.07	2	8.0	7.9	0.07	2	8.3	8.3	0.03	2
	5600	7.7	7.3	0.49	7	8.2	8.1	0.07	2	7.9	7.9	0.00	2	8.3	8.3	0.02	2
	10000	7.6	7.2	0.44	6	8.1	8.0	0.14	2	7.6	7.6	0.00	2	8.3	8.3	0.02	2
	Summary	7.8	7.2	0.55	64	8.3	8.0	0.15	18	8.2	7.6	0.29	19	8.4	8.3	0.07	18

		Chironomid 10:1				Hexagenia 4:1				Tubifex 10:1				Hyalella 67:1			
Site	NOM	Mean	Min	Std	N	Mean	Min	Std	N	Mean	Min	Std	N	Mean	Min	Std	N
Long	0	8.3	8.0	0.19	12	8.3	8.2	0.12	4	8.4	8.1	0.20	12	8.3	8.3	0.08	3
Point with	180	8.4	8.2	0.14	7	8.2	8.0	0.17	4	8.3	8.1	0.16	8	8.4	8.3	0.06	3
100%	320	8.4	8.3	0.08	8	8.3	8.2	0.06	4	8.3	8.1	0.19	8	8.4	8.3	0.08	3
DeChlor	560	8.3	8.3	0.04	8	8.3	8.2	0.06	3	8.2	8.1	0.14	8	8.4	8.3	0.06	3
Overlay	1000	8.3	8.3	0.05	8	8.3	8.1	0.17	4	8.3	8.1	0.18	8	8.4	8.2	0.10	3
Water	1800	8.3	8.3	0.00	7	8.2	8.2	0.00	4	8.2	8.0	0.19	8	8.4	8.4	0.04	3
	3200	8.3	8.3	0.00	8	8.2	8.2	0.00	3	8.2	8.0	0.17	8	8.4	8.3	0.08	3
	5600	8.3	8.1	0.07	8	8.2	8.2	0.00	4	8.2	8.0	0.14	6	8.4	8.3	0.08	3
	10000	8.2	8.1	0.05	7	8.1	8.0	0.06	4	8.1	7.9	0.12	8	8.4	8.3	0.03	3
	Summary	8.3	8.0	0.11	73	8.2	8.0	0.11	34	8.2	7.9	0.19	74	8.4	8.2	0.06	27

		Hyalella 67:1				Hyalella 200:1				Hyalella 500:1				Hyalella 1000:1			
Site	NOM	Mean	Min	Std	N	Mean	Min	Std	N	Mean	Min	Std	N	Mean	Min	Std	N
Restoule	0	8.1	8.1		1	6.1	5.5	0.97	2	7.2	7.1	0.24	2	7.3	6.5	1.1	2
with 10%	320	6.7	6.7		1	7.3	7.2	0.16	2	7.4	7.1	0.36	2	7.6	7.4	0.2	2
DeChlor	560	7.0	7.0		1	7.7	7.0	1.06	2	7.4	7.1	0.46	2	8.0	7.3	1.0	2
Overlay	1000	7.0	7.0		1	7.2	6.9	0.47	2	7.7	7.2	0.71	2	7.5	7.4	0.2	2
Water	1800	7.1	7.1		1	7.3	7.0	0.34	2	7.4	7.1	0.44	2	8.1	7.5	0.9	2
	3200	7.1	7.1		1	7.3	6.7	0.80	2	7.3	7.0	0.40	2	7.6	7.4	0.2	2
	5600	7.2	7.2		1	7.2	6.9	0.34	2	7.3	7.1	0.30	2	7.6	7.5	0.2	2
	10000	7.1	7.1		1	7.2	7.0	0.30	2	7.4	7.2	0.27	2	7.7	7.6	0.1	2
	18000	7.1	7.1		1	7.2	7.0	0.21	2	7.3	7.1	0.24	2	7.6	7.6	0.0	2
	Summary	7.2	6.7	0.37	9	7.2	5.5	0.60	18	7.4	7.0	0.32	18	7.7	6.5	0.5	18
Restoule	0	8.2	8.0	0.14	3	8.5	8.4	0.07	2	8.4	8.2	0.29	2	8.5	8.3	0.4	2
with 100%	320	8.1	8.1	0.06	4	8.4	8.4	0.06	2	8.4	8.3	0.22	2	8.4	8.4	0.1	2
DeChlor	560	8.1	7.9	0.12	3	8.4	8.4	0.08	2	8.5	8.2	0.41	2	8.4	8.3	0.1	2
Overlay	1000	8.2	8.1	0.08	3	8.4	8.4	0.01	2	8.4	8.3	0.18	2	8.4	8.4	0.0	2
Water	1800	8.2	8.1	0.07	3	8.4	8.4	0.03	2	8.6	8.3	0.45	2	8.5	8.4	0.1	2
	3200	8.2	8.1	0.09	3	8.5	8.4	0.06	2	8.4	8.2	0.24	2	8.7	8.5	0.3	2
	5600	8.1	8.1	0.08	3	8.4	8.4	0.04	2	8.6	8.6	0.08	2	8.4	8.4	0.1	2
	10000	8.1	8.0	0.05	3	8.4	8.3	0.05	2	8.3	8.2	0.16	2	8.4	8.4	0.0	2
	18000	7.9	7.8	0.09	3	8.4	8.3	0.03	2	8.3	8.2	0.18	2	8.4	8.4	0.1	2
	Summary	8.1	7.8	0.12	28	8.4	8.3	0.06	18	8.4	8.2	0.22	18	8.5	8.3	0.1	18

Appendix 2

Total Ammonia ($NH_3 + NH_4^+$ mmol•L^{-1}): mean, minimum, standard deviation and and sample size (N) of measured total ammonia in overlay waters from the different; sediment treatments, organism tests, water to sediment ratios and water hardness (Restoule only).

		Chironomid 10:1				Hexagenia 10:1				Tubifex (indigenous) 10:1				Hyalella 67:1			
Site	NOM	Mean	Max	Std	N	Mean	Max	Std	N	Mean	Max	Std	N	Mean	Max	Std	N
L. Erie	0	0.000	0.000	0.000	12	0.005	0.008	0.005	2	0.000	0.000	0.000	3	0.000	0.000	0.000	2
303 with	180	0.000	0.000	0.000	8	0.003	0.005	0.003	2	0.000	0.000	0.000	2	0.000	0.000	0.000	2
100%	320	0.000	0.000	0.000	8	0.002	0.004	0.003	2	0.000	0.000	0.000	2	0.000	0.000	0.000	2
DeChlor	560	0.000	0.000	0.000	8	0.002	0.003	0.002	2	0.000	0.000	0.000	2	0.000	0.000	0.000	2
Overlay	1000	0.000	0.000	0.000	8	0.002	0.004	0.003	2	0.000	0.000	0.000	2	0.000	0.000	0.000	2
Water	1800	0.000	0.000	0.000	8	0.002	0.004	0.003	2	0.000	0.000	0.000	2	0.000	0.000	0.000	2
	3200	0.240	0.371	0.153	8	0.002	0.003	0.002	2	0.382	0.382	0.000	2	0.060	0.060	0.000	2
	5600	0.365	0.382	0.026	8	0.006	0.008	0.004	2	0.382	0.382	0.000	2	0.105	0.120	0.021	2
	10000	0.347	0.382	0.061	7	0.007	0.012	0.006	2	0.441	0.441	0.000	2	0.090	0.090	0.000	2
	Summary	0.097	0.382	0.158	75	0.003	0.012	0.003	18	0.127	0.441	0.193	19	0.028	0.120	0.043	18

		Chironomid 10:1				Hexagenia 4:1				Tubifex 10:1				Hyalella 67:1			
Site	NOM	Mean	Max	Std	N	Mean	Max	Std	N	Mean	Max	Std	N	Mean	Max	Std	N
Long	0	0.000	0.000	0.000	12	0.007	0.009	0.002	4	0.000	0.000	0.000	12	0.000	0.000	0.000	3
Point with	180	0.000	0.000	0.000	7	0.005	0.007	0.003	4	0.000	0.000	0.000	8	0.000	0.000	0.000	3
100%	320	0.000	0.000	0.000	8	0.013	0.022	0.010	4	0.000	0.000	0.000	8	0.000	0.000	0.000	3
DeChlor	560	0.000	0.000	0.000	8	0.014	0.019	0.008	3	0.000	0.000	0.000	8	0.000	0.000	0.000	3
Overlay	1000	0.000	0.000	0.000	8	0.008	0.012	0.005	4	0.000	0.000	0.000	8	0.020	0.060	0.035	3
Water	1800	0.000	0.000	0.000	7	0.005	0.008	0.003	4	0.000	0.000	0.000	8	0.010	0.030	0.017	3
	3200	0.000	0.000	0.000	8	0.005	0.006	0.002	3	0.000	0.000	0.000	8	0.020	0.030	0.017	3
	5600	0.000	0.000	0.000	8	0.010	0.017	0.008	4	0.000	0.000	0.000	6	0.030	0.060	0.030	3
	10000	0.046	0.147	0.049	7	0.044	0.085	0.047	4	0.037	0.147	0.068	8	0.040	0.090	0.046	3
	Summary	0.004	0.147	0.020	73	0.012	0.085	0.019	34	0.004	0.147	0.024	74	0.013	0.090	0.024	27

		Hyalella 67:1				Hyalella 200:1				Hyalella 500:1				Hyalella 1000:1			
Site	NOM	Mean	Max	Std	N	Mean	Max	Std	N	Mean	Max	Std	N	Mean	Max	Std	N
Restoule	0	0.100	0.180	0.075	3	0.000	0.000	0.000	2	0.050	0.090	0.057	2	0.019	0.030	0.016	2
with 10%	320	0.210	0.210		1	0.060	0.120	0.085	2	0.020	0.030	0.014	2	0.015	0.030	0.021	2
DeChlor	560	0.210	0.210		1	0.000	0.000	0.000	2	0.018	0.030	0.017	2	0.015	0.030	0.021	2
Overlay	1000	0.210	0.210		1	0.060	0.120	0.085	2	0.016	0.030	0.020	2	0.046	0.090	0.063	2
Water	1800	0.210	0.210		1	0.045	0.090	0.064	2	0.030	0.060	0.042	2	0.000	0.000	0.000	2
	3200	0.210	0.210		1	0.030	0.060	0.042	2	0.034	0.060	0.037	2	0.050	0.090	0.057	2
	5600	0.210	0.210		1	0.015	0.030	0.021	2	0.019	0.030	0.016	2	0.045	0.090	0.064	2
	10000	0.210	0.210		1	0.015	0.030	0.021	2	0.030	0.060	0.042	2	0.030	0.060	0.042	2
	18000	0.210	0.210		1	0.015	0.030	0.021	2	0.030	0.060	0.042	2	0.030	0.060	0.042	2
	Summary	0.180	0.210	0.061	11	0.027	0.120	0.042	18	0.027	0.090	0.028	18	0.028	0.090	0.035	18
Restoule	0	0.030	0.060	0.030	3	0.000	0.000	0.000	2	0.000	0.000	0.000	2	0.000	0.000	0.000	2
with 100%	320	0.030	0.060	0.035	4	0.030	0.060	0.042	2	0.045	0.090	0.064	2	0.045	0.090	0.064	2
DeChlor	560	0.030	0.060	0.030	3	0.015	0.030	0.021	2	0.003	0.006	0.005	2	0.046	0.090	0.062	2
Overlay	1000	0.040	0.090	0.046	3	0.000	0.000	0.000	2	0.037	0.060	0.032	2	0.031	0.060	0.042	2
Water	1800	0.060	0.090	0.052	3	0.030	0.060	0.042	2	0.020	0.030	0.015	2	0.046	0.090	0.062	2
	3200	0.060	0.090	0.052	3	0.000	0.000	0.000	2	0.036	0.060	0.035	2	0.015	0.030	0.021	2
	5600	0.060	0.090	0.052	3	0.015	0.030	0.021	2	0.032	0.060	0.040	2	0.018	0.030	0.017	2
	10000	0.060	0.090	0.052	3	0.015	0.030	0.021	2	0.034	0.060	0.036	2	0.045	0.090	0.064	2
	18000	0.060	0.090	0.052	3	0.015	0.030	0.021	2	0.047	0.090	0.061	2	0.045	0.090	0.064	2
	Summary	0.047	0.090	0.040	28	0.013	0.060	0.021	18	0.028	0.090	0.032	18	0.032	0.090	0.040	18

Appendix 3

Oxygen (mg•L^{-1}): mean, minimum, standard deviation and and sample size (N) of measured oxygen in overlay waters from the different; sediment treatments, organism tests, water to sediment ratios and water hardness (Restoule only).

		Chironomid 10:1				Hexagenia 10:1				Tubifex (indigenous) 10:1				Hyalella 67:1			
Site	NOM	Mean	Min	Std	N	Mean	Min	Std	N	Mean	Min	Std	N	Mean	Min	Std	N
L. Erie	0	7.5	4.6	1.05	10	8.2	8.2	0.00	2	7.3	7.1	0.20	3	8.3	8.2	0.11	2
303 with	180	7.8	7.4	0.33	7	8.3	8.2	0.07	2	7.2	7.0	0.21	2	8.3	8.2	0.04	2
100%	320	7.8	7.4	0.30	7	8.2	8.1	0.07	2	7.3	7.3	0.00	2	8.2	8.1	0.11	2
DeChlor	560	7.8	7.5	0.25	7	8.3	8.2	0.07	2	7.3	7.2	0.07	2	8.1	8.1	0.04	2
Overlay	1000	7.7	7.3	0.27	7	8.3	8.3	0.00	2	7.5	7.4	0.14	2	8.1	8.1	0.07	2
Water	1800	7.7	7.4	0.30	7	8.4	8.3	0.07	2	7.4	7.1	0.35	2	8.1	8.0	0.14	2
	3200	7.7	7.4	0.19	7	8.3	8.2	0.07	2	8.0	7.9	0.07	2	8.1	7.9	0.26	2
	5600	7.7	7.3	0.31	7	8.0	7.8	0.28	2	7.9	7.9	0.00	2	8.1	7.9	0.20	2
	10000	7.8	7.5	0.22	6	8.2	8.0	0.21	2	7.6	7.6	0.00	2	8.1	8.0	0.12	2
	Summary	7.7	4.6	0.47	65	8.2	7.8	0.14	18	7.5	7.0	0.30	19	8.1	7.9	0.13	18

		Chironomid 10:1				Hexagenia 4:1				Tubifex 10:1				Hyalella 67:1			
Site	NOM	Mean	Min	Std	N	Mean	Min	Std	N	Mean	Min	Std	N	Mean	Min	Std	N
Long	0	7.9	7.4	0.26	12	9.1	8.8	0.29	4	8.1	7.8	0.21	12	8.3	8.0	0.24	3
Point with	180	7.9	7.4	0.27	7	8.3	7.7	0.64	4	7.9	7.7	0.12	8	8.2	8.0	0.14	3
100%	320	7.9	7.6	0.24	8	8.3	8.1	0.23	4	8.0	7.7	0.18	8	8.1	8.0	0.06	3
DeChlor	560	7.8	7.5	0.20	8	8.6	8.6	0.00	3	7.9	7.7	0.14	8	8.1	8.0	0.08	3
Overlay	1000	7.8	7.7	0.11	8	8.2	7.6	0.64	4	8.1	7.9	0.09	8	8.0	7.9	0.10	3
Water	1800	7.7	7.5	0.14	7	8.3	8.1	0.23	4	8.0	7.8	0.12	8	8.1	8.1	0.03	3
	3200	7.8	7.5	0.19	8	8.6	8.4	0.40	3	7.9	7.7	0.11	8	8.0	7.9	0.16	3
	5600	7.8	7.4	0.24	8	8.3	8.1	0.17	4	7.9	7.7	0.17	6	8.1	8.0	0.12	3
	10000	7.8	7.3	0.25	7	8.4	7.6	0.57	4	8.0	7.7	0.15	8	8.1	7.9	0.15	3
	Summary	7.8	7.3	0.22	73	8.4	7.6	0.46	34	8.0	7.7	0.16	74	8.1	7.9	0.13	27

		Hyalella 67:1				Hyalella 200:1				Hyalella 500:1				Hyalella 1000:1			
Site	NOM	Mean	Min	Std	N	Mean	Min	Std	N	Mean	Min	Std	N	Mean	Min	Std	N
Restoule	0	8.3	8.2	0.06	3	6.3	6.3		1	7.0	7.0		1	7.3	7.3		1
with 10%	320	8.3	8.3		1	6.9	6.9		1	7.7	7.7		1	7.7	7.7		1
DeChlor	560	8.3	8.3		1	7.6	7.6		1	7.9	7.9		1	7.8	7.8		1
Overlay	1000	8.3	8.3		1	6.8	6.8		1	8.5	8.5		1	7.8	7.8		1
Water	1800	8.3	8.3		1	6.9	6.9		1	8.1	8.1		1	8.1	8.1		1
	3200	8.2	8.2		1	7.7	7.7		1	7.3	7.3		1	7.2	7.2		1
	5600	8.3	8.3		1	5.8	5.8		1	7.2	7.2		1	7.3	7.3		1
	10000	8.3	8.3		1	7.0	7.0		1	7.5	7.5		1	7.6	7.6		1
	18000	8.1	8.1		1	6.8	6.8		1	7.7	7.7		1	7.7	7.7		1
	Summary	8.3	8.1	0.07	11	6.9	5.8	0.60	9	7.6	7.0	0.45	9	7.6	7.2	0.28	9
Restoule	0	8.5	8.5	0.07	3	6.5	6.5		1	8.3	8.3		1	8.4	8.4		1
with 100%	320	8.4	8.3	0.08	4	6.9	6.9		1	7.9	7.9		1	7.9	7.9		1
DeChlor	560	8.3	8.2	0.17	3	6.9	6.9		1	8.4	8.4		1	8.4	8.4		1
Overlay	1000	8.3	8.1	0.11	3	7.1	7.1		1	7.5	7.5		1	7.6	7.6		1
Water	1800	8.2	8.1	0.11	3	7.2	7.2		1	8.9	8.9		1	8.7	8.7		1
	3200	8.2	8.1	0.15	3	6.7	6.7		1	7.8	7.8		1	7.9	7.9		1
	5600	8.3	8.2	0.09	3	6.6	6.6		1	8.0	8.0		1	8.0	8.0		1
	10000	8.3	8.1	0.13	3	7.1	7.1		1	7.9	7.9		1	8.0	8.0		1
	18000	8.3	8.1	0.13	3	6.1	6.1		1	7.8	7.8		1	7.8	7.8		1
	Summary	8.3	8.1	0.14	28	6.8	6.1	0.42	9	8.0	7.5	0.42	9	8.1	7.6	0.35	9

Appendix 4

Conductivity ($\mu S \cdot cm^{-1}$): mean, maximum, standard deviation and and sample size (N) of overlay waters from each nominal (NOM) sediment concentation with the different; spiked sediments, organisms, water:sediment ratios and water hardness (Restoule only).

		Chironomid 10:1				Hexagenia 10:1				Tubifex (indigenous) 10:1				Hyalella 67:1			
Site	NOM	Mean	Max	Std	N	Mean	Max	Std	N	Mean	Max	Std	N	Mean	Max	Std	N
L. Erie	0	545	600	67.2	10	540	554	20.5	2	510	530	26	3	444	444	0.7	2
303 with	180	554	590	19.0	7	491	512	29.7	2	455	480	35	2	410	433	33.2	2
100%	320	557	580	16.0	7	462	488	36.8	2	460	540	113	2	417	448	44.5	2
DeChlor	560	461	580	169	7	446	476	42.4	2	590	620	42	2	404	434	43.1	2
Overlay	1000	500	620	169	7	530	562	46.0	2	650	880	325	2	429	446	24.7	2
Water	1800	720	770	33.7	7	550	575	36.1	2	1075	1110	49	2	432	462	43.1	2
	3200	854	890	28.2	7	688	729	58.0	2	2048	2130	116	2	438	479	58.0	2
	5600	1117	1200	71.1	7	867	901	48.8	2	2625	2730	148	2	493	511	26.2	2
	10000	1667	1840	170.8	6	1207	1244	52.3	2	3990	4250	368	2	571	606	50.2	2
	Summary	751	1840	366.8	65	642	1244	243.8	18	1332	4250	1203	19	448	606	58.6	18

		Chironomid 10:1				Hexagenia 4:1				Tubifex 10:1				Hyalella 67:1			
Site	NOM	Mean	Max	Std	N	Mean	Max	Std	N	Mean	Max	Std	N	Mean	Max	Std	N
Long	0	521	581	36.7	12	484	508	28.3	4	451	480	27.1	12	392	429	34.8	3
Point with	180	550	664	73.0	7	460	466	6.9	4	441	470	23.6	8	381	413	27.5	3
100%	320	550	642	57.1	8	418	460	48.5	4	456	480	13.0	8	395	420	21.4	3
DeChlor	560	532	590	36.4	8	459	469	16.7	3	478	530	48.0	8	397	427	27.8	3
Overlay	1000	469	614	179.6	8	446	464	20.8	4	444	470	22.0	8	399	431	35.3	3
Water	1800	581	656	48.3	7	459	481	26.0	4	481	520	32.3	8	393	426	28.7	3
	3200	636	719	49.7	8	500	510	17.3	3	521	560	23.0	8	400	428	25.0	3
	5600	761	933	92.5	8	554	585	35.8	4	638	730	67.9	6	420	451	30.1	3
	10000	947	1179	130.6	7	638	674	42.1	4	821	920	57.9	8	427	456	25.7	3
	Summary	608	1179	160.1	73	491	674	70.9	34	519	920	123.5	74	400	456	27.6	27

		Hyalella 67:1				Hyalella 200:1				Hyalella 500:1				Hyalella 1000:1			
Site	NOM	Mean	Max	Std	N	Mean	Max	Std	N	Mean	Max	Std	N	Mean	Max	Std	N
Restoule	0	89	104	18.9	3	61.2	76.1	21.1	2	52.5	62.1	13.6	2	50.5	55.0	6.4	2
with 10%	320	89	89		1	58.5	70.6	17.1	2	46.1	50.9	6.9	2	45.3	50.4	7.3	2
DeChlor	560	87	87		1	47.5	51.7	5.9	2	48.5	53.1	6.6	2	46.9	51.6	6.6	2
Overlay	1000	107	107		1	62.8	77.3	20.6	2	48.8	55.9	10.0	2	53.7	65.8	17.2	2
Water	1800	107	107		1	61.5	75.1	19.2	2	57.7	70.7	18.4	2	49.1	54.8	8.1	2
	3200	117	117		1	56.6	60.8	6.0	2	59.4	71.9	17.7	2	57.5	70.3	18.2	2
	5600	129	129		1	68.1	78.6	14.8	2	57.9	63.2	7.5	2	55.2	65.5	14.6	2
	10000	173	173		1	86.3	106.7	28.9	2	65.7	76.7	15.6	2	55.3	63.1	11.0	2
	18000	228	228		1	96.5	106.9	14.7	2	71.4	79.5	11.5	2	59.0	64.9	8.3	2
	Summary	119	228	45.2	11	66.5	106.9	19.8	18	56.4	79.5	12.4	18	52.5	70.3	9.7	18

Site	NOM	Mean	Max	Std	N	Mean	Max	Std	N	Mean	Max	Std	N	Mean	Max	Std	N
Restoule	0	342	360	26.7	3	357	368	16.3	2	380	406	36.8	2	349	360	16.3	2
with 100%	320	336	352	23.1	4	368	388	29.0	2	398	431	46.7	2	360	378	26.2	2
DeChlor	560	333	347	19.3	3	390	424	48.8	2	347	352	7.8	2	368	389	30.4	2
Overlay	1000	345	377	30.6	3	357	371	19.8	2	369	391	31.1	2	351	358	10.6	2
Water	1800	343	356	19.7	3	340	347	9.9	2	368	380	17.7	2	373	403	42.4	2
	3200	359	373	23.1	3	379	405	36.8	2	388	414	37.5	2	354	365	16.3	2
	5600	360	366	8.1	3	374	395	30.4	2	375	407	45.3	2	353	362	13.4	2
	10000	382	403	21.5	3	379	403	33.9	2	371	389	25.5	2	352	362	14.8	2
	18000	409	420	9.9	3	378	395	24.0	2	367	381	19.8	2	380	406	37.5	2
	Summary	356	420	29.7	28	369	424	26.2	18	374	431	27.3	18	360	406	21.5	18

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